Synthesis of Isomeric Analogues of Coenzyme Pyrroloquinoline Quinone (PQQ)

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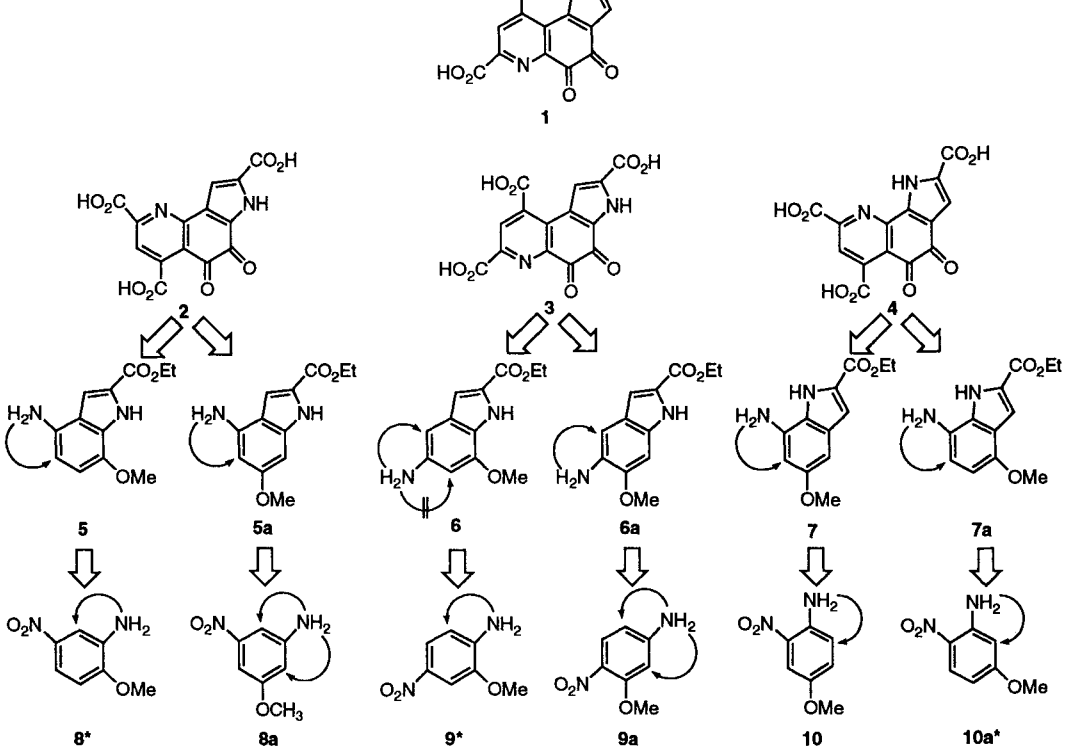
Three isomeric analogues **2–4** of the redox-active coenzyme 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid (**1**, PQQ, methoxatin) were synthesized from methoxynitroanilines **8**, **9** and **10a**, respectively. Reaction of the diazonium salts of each of the starting compounds with ethyl α-methylacetoacetate gave the corresponding substituted phenylhydrazones of ethyl pyruvate. These intermediates underwent acid-catalyzed Fischer indolization and gave the esters **13**, **17** and **25**, respectively. Reduction to the corresponding aminoindoles with hydrogen over Pd/C, followed by Doebner-von Miller quinoline synthesis with dimethyl *trans*-2-ketoglutaconate, oxidation of the intermediate methoxy compound to the *o*-quinone, and hydrolysis of triester products gave **2**, **3**, and **4**, respectively. These isomers will serve as authentic examples to define their possible formation in nature and will also serve as isosteric probes to define the binding of PQQ at active sites in PQQ-requiring quinoproteins.

Pyrroloquinoline quinone (**1**, PQQ, methoxatin, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid) was recognized as a novel coenzyme for methanol dehydrogenase of the methylotrophic soil bacterium *Pseudomonas sp.* in 1979.[1] Since then, many quinoproteins which use quinonoid compounds as cofactors have been reported.[2,3] An important role has been suggested for PQQ and its closely related analogues as growth or nutritional factors at critical stages in eukaryotic development.[4] Hultquist observed that PQQ can react with erythrocyte flavin reductase to reduce ferryl myoglobin in vitro and protect isolated rabbit heart from reoxygenation injury.[5] This observation suggested that PQQ may act as a tissue-protective agent. In addition to its nutritional and enzymatic importance, PQQ and its derivatives have been reported to inhibit reverse transcriptase,[6] lower rat blood and liver acetaldehyde by an accelerated oxidation of acetaldehyde,[7] prevent cyclohexiimide-induced memory disturbance in mice,[8] stimulate the growth of microbial, plant and animal cells in culture,[9] possess hair growth-stimulating activity[10] and inhibit aldose reductase activity.[11] These activities suggest numerous therapeutic possibilities for PQQ and its analogues.

While PQQ is a known redox-active cofactor in some quinoproteins, both PQQ and its isomeric PQQ analogues may be formed both enzymatically and as breakdown products derived from the turnover of topaquinone-containing amine oxidases. The formation of isomer **3** has been postulated but not confirmed.[3,12] In addition to **3**, two other isomeric analogues of PQQ, **2** and **4**, may be formed in nature as a result of nonenzymatic processes



Scheme 1 * preferred starting material

associated with quinoprotein turnover, as well as, perhaps through direct enzymatic reactions which remain to be characterized. We report here the syntheses of all three aza isomers **2**–**4** of PQQ. In a previous communication,[13] we demonstrated a clean separation of isomers **2** and **3** from PQQ by HPLC, but not isomer **4**. These compounds will serve as authentic samples to define their possible formation in nature,[3,12] and will also serve as isosteric probes to define the binding of PQQ at active sites in PQQ-requiring quinoproteins. In addition, these isomeric PQQs may be used as nonenzymatic antioxidants or as redox-active cofactors in enzyme systems which remain to be defined.[5] They may further establish whether vitamin or vitamin-like effects of PQQ in mammalian systems are enzymatically or nonenzymatically mediated.[4]

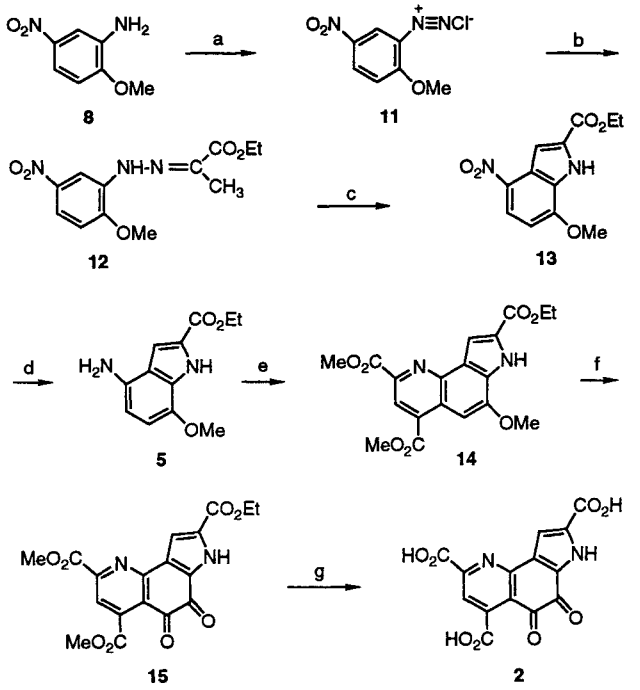
Corey's elegant synthesis of PQQ[14] appeared to be a versatile method of general applicability for the synthesis of all three isomeric analogues of PQQ from trisubstituted benzene derivatives via Fischer indolization followed by Doebner–von Miller quinoline synthesis. However, instead of using a singly protected diaminobenzene derivative we successfully utilized an aminonitro derivative, thus eliminating the need for protection and deprotection of the amino function. While this work was in progress Martin and Winkler[15] reported the syntheses of the triesters of the three target molecules, where a different approach was used for the synthesis of different indole intermediates. Our approach was based on the use of different positional isomers of methoxynitroanilines for the synthesis of the required indole intermediates.

Retrosynthetic alternatives for each PQQ isomer were considered through two isomeric indoles and two trisubstituted benzene derivatives (Scheme 1). In the synthetic direction, the choice of benzene derivatives to form the appropriate indole was based primarily on the availability or ease of synthesis of the benzene derivatives and on the likelihood that each of the cyclization reactions (Fischer indole synthesis and Doebner–von Miller quinoline synthesis) would proceed to give a single product.

Commercially available 2-methoxy-5-nitroaniline **(8)** was converted to the hydrazone **12** via the diazonium salt **11** by Japp–Klingemann reaction (Scheme 2). Attempts to cyclize **12** to the indole **13** in formic acid or in HCl-saturated ethanol were unsuccessful and led to the *syn*-isomer of **12**. Cyclization was finally accomplished in polyphosphoric acid. Doebner–von Miller reaction of the aminoindole **5** with dimethyl *trans*-2-ketoglutaconate gave the pyrroloquinoline **14**. Oxidation of **14** with ceric ammonium nitrate (CAN) in $MeCN/H_2O$ (5:1) afforded the pyrroloquinoline quinone triester **15**, which was hydrolyzed in 0.5 M aqueous K_2CO_3 solution at 60 °C for 8 hours to yield **2**.

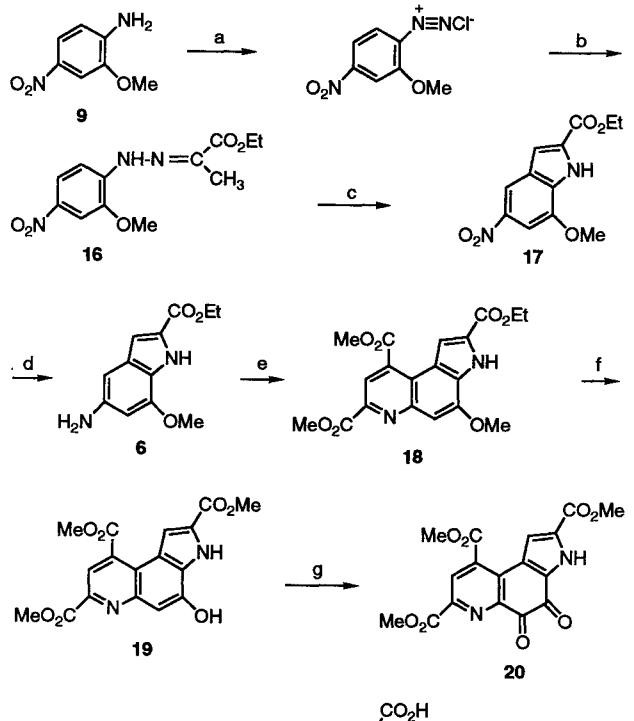
Commercially available 2-methoxy-4-nitroaniline **(9)** was preferred to 3-methoxy-4-nitroaniline **(9a)** as the starting material for the synthesis of **3** in view of the likelihood of obtaining a single product in Fischer indole synthesis (cf., Scheme 1). However, the corresponding hydrazone **16** failed to cyclize in polyphosphoric acid, formic acid, HCl-saturated ethanol or with $ZnCl_2$ in cumene or xylene. This was finally accomplished with $ZnCl_2$ in nitrobenzene at 160–180 °C (Scheme 3). As predicted on steric grounds, the aminoindole **6** reacted with dimethyl *trans*-2-ketoglutaconate with cyclization occurring selectively at the 4-position to form the angular pyrroloquinoline **18** rather than at the alternate 6-position to form the corresponding linear isomer. The angular structure of the product **18** was confirmed by 2D-NMR spectroscopy (NOESY) in which an NOE was observed between the aromatic proton at C-5 and the methoxy protons at C-4, consistent with a similar NOE observed between the corresponding protons in the indole **17**. The pyrroloquinoline **18** could not be directly oxidized to the *o*-quinone **20** using ceric ammonium nitrate or silver oxide. However, after demethylation with HBr (33%) in AcOH and esterification with $MeOH/H^+$, the hydroxyquinoline trimethyl ester **19** was obtained and was oxidized with Fremy's salt[16] to obtain the *o*-quinone **20**, which was hydrolyzed to the final product **3**.

Either of the two compounds 4-methoxy-2-nitroaniline **(10)** or 5-methoxy-2-nitroaniline **(10a)** appeared to be equally suitable starting materials for the synthesis of **4** (cf., Scheme 1). Our initial approach using commercially available **10** as the starting material proved unsuccessful. The failure of this approach prompted us to use 5-methoxy-2-nitroaniline **(10a)** as the starting material (Scheme 4). **10a** was not commercially available and was prepared from *m*-anisidine **21** by acetylation, followed by nitration



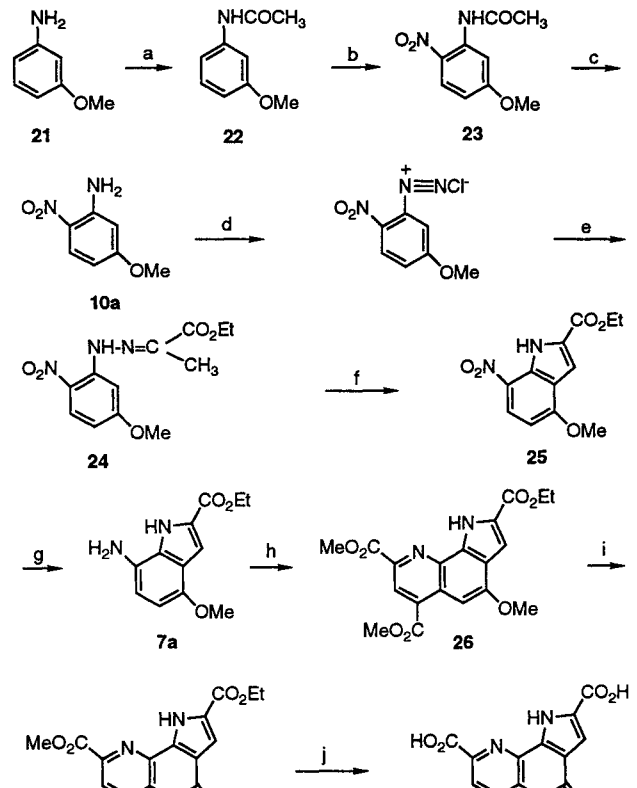
a) $NaNO_2$, HCl, H_2O, 0-5 °C, 20 min. b) ethyl α-methyl acetoacetate, KOH, EtOH, H_2O, 0 °C, 1 h, 88%. c) polyphosphoric acid, 100 °C, 10 h, 23%. d) H_2, Pd-C, EtOH, r.t., 2 h, 90%. e) (i) dimethyl trans-2-ketoglutaconate, CH_2Cl_2, r.t., 15h; (ii) dry HCl, O_2, r.t., 6h, 77%. f) CAN, MeCN, H_2O, 0-2 °C, 1.5 h, 50%. g) (i) K_2CO_3, 60 °C, 8 h; (ii) HCl, r.t., 99%.

Scheme 2



a) $NaNO_2$, HCl, H_2O, 0 °C, 30 min. b) ethyl α-methyl acetoacetate, KOH, EtOH, H_2O, 0 °C, 1 h, 63%. c) $ZnCl_2$, nitrobenzene, 160-180 °C, 8 h, 8%. d) H_2, Pd-C, EtOH, r.t., 3 h, 98%. e) (i) dimethyl *trans*-2-ketoglutaconate, CH_2Cl_2, r.t., 15 h; (ii) dry HCl, O_2, r.t., 9 h, 82%. f) (i) HBr in AcOH, N_2, 50-115 °C, 22h; (ii) MeOH, H^+, reflux, 20 h, 78%. g) Fremy's salt, MeCN, H_2O, 0-5 °C, 12 h, 68%. h) (i) K_2CO_3 ,60 °C, 10 h; (ii) HCl, r.t., 92%.

Scheme 3



a) Ac_2O, HCl, NaOAc, H_2O, r.t., 20 min, 91%. b) HNO_3, Ac_2O, 25-45 °C, 6 h, 63%. c) HCl, H_2O, reflux, 5 h, 85%. d) $NaNO_2$, HCl, H_2O, 0-5 °C, 1 h. e) ethyl α-methyl acetoacetate, KOH, EtOH, H_2O, 2-5 °C, 1 h, 62%. f) polyphosphoric acid, 100 °C, 8.5 h, 23%. g) H_2, Pd-C, EtOH, r.t., 97%. h) (i) dimethyl trans-2-ketoglutaconate, CH_2Cl_2, r.t., 12 h; (ii) dry HCl, O_2, r.t., 10 h, 77%. i) CAN, MeCN, H_2O, 0-2 °C, 3.5 h, 79%. j) (i) K_2CO_3, 60 °C, 10 h; (ii) HCl, r.t., 91%.

Scheme 4

and hydrolysis. *o*-Nitroacetanilide derivative **23** has been previously obtained by the nitration of **22** at low temperature and separation of the *o*-nitro derivative from the para-isomer by extraction with ligroin. We observed that the *o*-nitro derivative **23** could be obtained as the major product when the reaction was carried out at higher temperatures. The overall yield of the product was about the same in either method. Using the Japp–Klingemann reaction, **10a** was converted to arylhydrazone **24**. The aminoindole **7a**, prepared by Fischer indolization of **24**, followed by hydrogenation, cleanly underwent Doebner–von Miller cyclization with dimethyl *trans*-2-ketoglutaconate to form the pyrroloquinoline **26**. Oxidation of **26** with CAN directly gave the pyrroloquinoline quinone triester **27**. Quinone **4** was obtained by the hydrolysis of **27** with 0.5 M aqueous K_2CO_3 solution.

In our approach, the precursor triesters of **2**, **3** and **4** were obtained in a fewer number of steps and in higher overall yields compared to published methods.[15] Thus, in the synthesis of **2**, the corresponding triester **15** was obtained in 5 steps and in an overall yield of 7.01% (in comparison to 8 steps and an overall yield of 0.21% or 13 steps and an overall yield of 1.03% in procedures reported by Martin and Winkler[15]). Compound **20**, the precursor triester of **3**, was obtained in 6 steps and in 2.15% overall yield (in comparison to 12 steps and in 0.24% overall yield[15]). Compound **27**, the precursor triester of **4**, was obtained in 8 steps and in 4.10% overall yield (in comparison to 8 steps and in 2.4% overall yield[15]).

NMR spectra were obtained on a Bruker AC-F 300 MHz instrument. Chemical shifts were expressed in ppm downfield from internal TMS or residual signal of deuterated solvent. For ^{13}C NMR, attached proton tests (APT) were performed to distinguish different carbons. IR spectra were measured on a Nicolet 5SFT-IR spectrophotometer. Starting materials were purchased from Aldrich, Pfalfz & Bauer, TCI America, and were used without purification. Column chromatography was conducted using Davisil silica gel (230–425 mesh, Fisher), Pre-coated silica gel plastic sheets (Art. 5735, E. Merck) were used in TLC. Melting points were uncorrected. Elemental analyses were performed by the Instrumentation Center, College of Arts and Sciences, University of Toledo. Satisfactory elemental analyses obtained for all new compounds: C ± 0.37, H ± 0.26, N ± 0.46 (exception: **2**, C + 0.5). Compound **2**, **3** and **4** contained 3.5, 3.0 and 2.5 parts of occluded H_2O. Dimethyl *trans*-2-

ketoglutaconate was prepared freshly from dimethyl 2-ketoglutarate before use.[14]

Ethyl Pyruvate 2-Methoxy-5-nitrophenylhydrazone (12):
To a mixture of 2-methoxy-5-nitroaniline (**8**; 98%, 8.6 g, 0.05 mol) and 4 M HCl solution (62.5 mL) maintained at 0–5 °C was slowly added a solution of $NaNO_2$ (3.5 g, 0.05 mol) in H_2O (10 mL). After stirring at 0–5 °C for 20 min, the resulting diazonium salt solution was added into a mixture of ethyl α-methyl acetoacetate (90%, 7.97 g, 0.05 mol), EtOH (6.0 mL), KOH (1.5 g, 0.27 mol), NaOAc (15 g) and H_2O (85 mL), and stirred at 0 °C for 1 h. The precipitate formed was collected by filtration and washed well with water to obtain **12** as a yellowish brown solid (12.4 g, 88%). An analytical sample was obtained by recrystallization twice from EtOH; mp 165–167 °C. R_f 0.60 (2% MeOH in CH_2Cl_2).

IR (KBr): ν = 3320, 1684, 1561, 1221 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.41 (t, 3 H, J = 7.11 Hz), 2.15 (s, 3 H), 4.02 (s, 3 H), 4.35 (q, 2 H, J = 7.11 Hz), 6.91 (d, 1 H, J = 9.9 Hz), 7.85 (dd, 1 H, J = 9.9, 2.7 Hz), 8.07 (br, 1 H), 8.38 (d, 1 H, J = 2.7 Hz).

^{13}C NMR ($CDCl_3$): δ = 10.62, 14.27, 56.36, 61.51, 108.75, 109.37, 117.26, 133.06, 136.10, 142.48, 150.43, 164.87.

Ethyl 7-Methoxy-4-nitroindole-2-carboxylate (13):
Polyphosphoric acid (200 g) was mixed carefully with the phenylhydrazone **12** (10 g, 0.036 mol) in toluene (170 mL). The mixture was stirred at 100 °C for 10 h. After cooling to r.t., the toluene layer was separated, and the polyphosphoric acid layer was extracted with toluene (3 × 100 mL). The combined toluene extracts were washed with aq $NaHCO_3$ solution, water, dried (Na_2SO_4), and evaporated in vacuo. The crude product was recrystallized from EtOH to afford yellow crystals (2.12 g, 23%); mp 205–206 °C; R_f 0.64 (2% MeOH in CH_2Cl_2).

IR (KBr): ν = 3289, 1715, 1499, 1345, 1252 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.45 (t, 3 H, J = 7.04 Hz), 4.09 (s, 3 H), 4.45 (q, 2 H, J = 7.04 Hz), 6.74 (d, 1 H, J = 9.7 Hz), 7.85 (d, 1 H, J = 2.7 Hz), 8.21 (d, 1 H, J = 9.7 Hz), 9.41 (br, 1 H).

^{13}C NMR ($CDCl_3$): δ = 14.31, 56.26, 61.63, 102.81, 108.86, 121.71, 122.60, 127.93, 130.13, 135.27, 151.75, 161.20.

Ethyl 4-Amino-7-methoxyindole-2-carboxylate (5):
A suspension of **13** (2.0 g, 7.58 mmol) in EtOH (80 mL) containing 10% Pd/C (0.1 g) was hydrogenated at 30 psi for 2 h. The catalyst was removed by filtration and the filtrate evaporated to dryness. The residue on recrystallization from $CHCl_3$ gave pale brown needles of **5** (1.6 g, 90%); mp 158–160 °C (dec); R_f 0.62 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3404, 3342, 1705, 1289 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.42 (t, 3 H, J = 7.12 Hz), 3.74 (br, 2 H), 3.88 (s, 3 H), 4.39 (q, 2 H, J = 7.12 Hz), 6.28 (d, 1 H, J = 8.9 Hz), 6.55 (d, 1 H, J = 8.9 Hz), 7.17 (d, 1 H, J = 2.2 Hz), 9.03 (br, 1 H).

^{13}C NMR ($CDCl_3$): δ = 14.41, 55.80, 60.88, 103.61, 105.60, 105.80, 119.50, 126.11, 128.79, 134.18, 139.88, 161.60.

2,4-Dimethoxycarbonyl-8-ethoxycarbonyl-6-methoxy-7H-pyrrole-[2,3-*h*]quinoline (14):
A mixture of **5** (0.672 g, 2.87 mmol) and dimethyl *trans*-2-ketoglutaconate[14] (0.6174 g, 3.5897 mmol) in CH_2Cl_2 (30 mL) was stirred at r.t. for 15 h. Additional CH_2Cl_2 (70 mL) was added and dry HCl gas passed through the reaction mixture for 3 h, after which both O_2 and HCl gases were passed for an additional 6 h. The mixture was poured into 0.1 M aq $NaHCO_3$ solution (100 mL) and CH_2Cl_2 (250 mL) was added. The organic layer was separated, washed with water (3 × 100 mL) and dried (Na_2SO_4). After evaporation of solvent, the residue was washed with EtOH to obtain compound **14** as a yellow solid (0.858 g, 77%); mp 284–285 °C; R_f 0.61 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3290, 1715, 1705, 1252 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.44 (t, 3 H, J = 7.13 Hz), 4.06 (s, 3 H), 4.10 (s, 3 H), 4.16 (s, 3 H), 4.44 (q, 2 H, J = 7.13 Hz), 8.12 (d, 1 H, J = 2.6 Hz), 8.19 (s, 1 H), 8.71 (s, 1 H), 9.56 (br, 1 H).

^{13}C NMR ($CDCl_3$): δ = 14.38, 52.71, 52.98, 55.95, 61.20, 98.39, 110.20, 121.62, 125.52, 125.93, 126.97, 129.25, 133.08, 142.76, 143.61, 150.20, 161.47, 165.91, 166.81.

2,4-Dimethoxycarbonyl-8-ethoxycarbonyl-7*H*-pyrrolo[2,3-*h*]quinoline-5,6-dione (15):
To a solution of **14** (772 mg, 2 mmol) in MeCN/H_2O (5:1, 100 mL) maintained at 0–2 °C was gradually added CAN (5.756 g, 10.5 mmol). After stirring for 0.5 h, additional 2.467 g (4.5 mmol) of CAN was added. The mixture was stirred for additional 1 h. Water (400 mL) was added, and the mixture was extracted with EtOAc/CH_2Cl_2 (4:1, 3 × 80 mL). After evaporation of solvent, the residue was recrystallized from $CHCl_3$ to afford yellow crystals (389 mg, 50%); mp 264–265 °C (Lit.[15] mp 262–263 °C); R_f 0.50 (5% MeOH in CH_2Cl_2).

5,6-Dihydro-5,6-dioxo-7*H*-pyrrolo[2,3-*h*]quinoline-2,4,8-tricarboxylic Acid (2):
Compound **15** (39 mg, 0.1 mmol) was suspended in 0.5 M aq K_2CO_3 solution (10 mL) and stirred at 60 °C for 8 h. The solution was acidified with 5 M HCl solution. The precipitate was collected by centrifugation, washed 2 times with water to give compound **2** as a brick-red solid (33 mg, 99%); mp > 300 °C (dec).

IR (KBr): ν = 3405 (br), 1735, 1719, 1702 cm^{-1}.

^1H NMR (DMSO-d_6): δ = 7.28 (s, 1 H), 7.67 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 111.38, 120.08, 122.44, 131.06, 131.25, 133.20, 146.77, 151.94, 152.67, 161.23, 165.16, 168.25, 168.37, 179.24.

Ethyl Pyruvate 2-Methoxy-4-nitrophenylhydrazone (16):
To a well stirred suspension of 2-methoxy-4-nitroaniline (**9**; 43 g, 0.25 mol, 98%) in 4 M HCl solution (312.5 mL, 1.25 mol) was slowly added a solution of $NaNO_2$ (17.5 g, 0.25 mol) in water (50 mL). After stirring at 0 °C for 30 min, the resulting diazonium salt solution was added into a vigorously stirred mixture of ethyl 2-methylacetoacetate (90%, 40 g, 0.25 mol), EtOH (300 mL), KOH (75 g, 1.35 mol), NaOAc (75 g) and H_2O (430 mL) maintained at 0 °C. The mixture was stirred continuously at 0 °C for 1 h. The precipitate was collected by filtration and washed with water to obtain **16** as a brown solid (45.2 g, 63%). An analytical sample was obtained by recrystallization twice from EtOH; mp 139–140 °C. R_{f1} 0.44, R_{f2} 0.72 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3350, 1710, 1596, 1532, 1322, 1275 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.40 (t, 3 H, J = 7.07 Hz), 2.18 (s, 3 H), 4.01 (s, 3 H), 4.35 (q, 2 H, J = 7.07 Hz), 7.63 (d, 1 H, J = 9.9 Hz), 7.76 (d, 1 H, J = 2.5 Hz), 7.96 (dd, 1 H, J = 9.9, 2.5 Hz), 8.32 (br, 1 H).

^{13}C NMR ($CDCl_3$): δ = 10.77, 14.27, 56.24, 61.67, 105.81, 112.22, 118.72, 137.70, 138.27, 141.41, 145.05, 164.67.

Ethyl 7-Methoxy-5-nitroindole-2-carboxylate (17):
A solution of **16** (44 g, 0.1566 mol) in redistilled nitrobenzene (400 mL) was heated with anhyd $ZnCl_2$ (44 g) at 160–180 °C for 8 h. Nitrobenzene was distilled off in vacuo and the residue was extracted with CH_2Cl_2 (6 × 200 mL). The combined CH_2Cl_2 extracts were passed through a small column of silica gel to remove the polar impurities. The filtrate was evaporated to dryness, and the residue was washed with hexane to remove the traces of nitrobenzene. The residue was recrystallized from EtOH to obtain light yellow crystals (3.17 g, 8%); mp 198–199 °C; R_f 0.52 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3290, 1698, 1526, 1347, 1322, 1260 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.44 (t, 3 H, J = 7.09 Hz), 4.07 (s, 3 H), 4.44 (q, 2 H, J = 7.09 Hz), 7.35 (d, 1 H, J = 2.4 Hz), 7.61 (d, 1 H, J = 1.9 Hz), 8.34 (d, 1 H, J = 1.9 Hz), 9.36 (br, 1 H).

^{13}C NMR ($CDCl_3$): δ = 14.32, 56.06, 61.56, 99.26, 110.68, 112.99, 126.58, 129.93, 130.68, 143.26, 146.04, 160.94.

Ethyl 5-Amino-7-methoxyindole-2-carboxylate (6):
A mixture of **17** (3.10 g, 11.74 mmol) in EtOH (250 mL) was hydrogenated over 10% Pd/C (250 mg) at 30 psi for 3 h. The catalyst was filtered and the filtrate evaporated to dryness at r.t. in vacuo to give **6** as a brown solid (2.7 g, 98%); R_f 0.22 (2% MeOH in

CH_2Cl_2). The product was used in the subsequent reaction without further purification.

7,9-Dimethoxycarbonyl-2-ethoxycarbonyl-4-methoxy-3*H*-pyrrole-[3,2-*f*]quinoline (18):
A mixture of **6** (2.7 g, 11.54 mmol) and dimethyl *trans*-2-ketoglutaconate[14] (2.48 g, 14.42 mmol) in anhyd CH_2Cl_2 (300 mL) was stirred at r.t. for 15 h. Additional CH_2Cl_2 (100 mL) was added and dry HCl gas was passed through the reaction mixture for 3 h, followed by both O_2 and dry HCl gases for additional 6 h. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 and was washed with water (2 × 100 mL), 0.2 M aq $NaHCO_3$ solution (100 mL) and water (2 × 100 mL), and dried (Na_2SO_4). The product was purified by passing through a pad of silica gel using MeOH/CH_2Cl_2 (1:9) as eluent and recrystallized from EtOH to give light yellow crystals (3.67 g, 82%); mp 219–220°C. R_f 0.42 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3303, 1711, 1260 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.45 (t, 3H, J = 7.12 Hz), 4.09 (s, 3H), 4.13 (s, 3H), 4.15 (s, 3H), 4.45 (q, 2H, J = 7.12 Hz), 7.51 (s, 1H), 7.61 (d, 1H, J = 2.6 Hz), 8.31 (s, 1H), 9.64 (br, 1H).

^{13}C NMR ($CDCl_3$): δ = 14.40, 53.19, 56.13, 61.32, 105.04, 111.51, 118.18, 119.01, 119.75, 126.73, 129.28, 136.22, 144.43, 148.49, 150.18, 161.10, 165.50, 168.63.

4-Hydroxy-2,7,9-trimethoxycarbonyl-3*H*-pyrrolo[3,2-*f*]quinoline (19):
A solution of **18** (2.0 g, 5.18 mmol) in HBr solution (33% HBr in AcOH 400 mL) was heated at 50°C for 5 h, at 80°C for 5 h, and then at 115°C for 12 h. After evaporation of solvent, the residue was refluxed with MeOH (400 mL) and concd H_2SO_4 (2 drops) for 20 h. MeOH was removed by distillation and the residue was washed with $CHCl_3$ (3 × 3 mL) to afford **19** as a light yellow solid (1.45 g, 78%); mp 276–278°C (dec.); R_f 0.28 (5% MeOH in CH_2Cl_2). An analytical sample was obtained by recrystallization from MeOH.

IR (KBr): ν = 3300, 1700, 1264 cm^{-1}.

^1H NMR (DMSO-d_6): δ = 3.88 (s, 3H), 3.93 (s, 3H), 4.05 (s, 3H), 7.24 (s, 1H), 7.39 (s, 1H), 8.07 (s, 1H).

^{13}C NMR (DMSO-d_6): δ = 51.97, 52.54, 53.28, 106.66, 110.61, 115.60, 116.88, 119.23, 126.94, 129.98, 135.83, 143.96, 148.31, 149.28, 160.87, 164.98, 168.35.

2,7,9-Trimethoxycarbonyl-3*H*-pyrrolo[3,2-*f*]quinoline-4,5-dione (20):
To a suspension of **19** (400 mg, 1.12 mmol) in MeCN/H_2O (5:1, 250 mL) maintained at 0–5°C was added a solution of Fremy's salt (600 mg, 2.24 mmol) and KH_2PO_4 (290 mg, 2.126 mmol) in H_2O (25 mL). The mixture was stirred at r.t. for 12 h, and H_2O (1000 mL) was added. The precipitate was collected by filtration and recrystallized from $CHCl_3$ to obtain **20** as yellow crystals (282 mg, 68%); mp 251–252°C (Lit.[15] mp > 250°C). R_f 0.46 (5% MeOH in CH_2Cl_2).

4,5-Dihydro-4,5-dioxo-3*H*-pyrrolo[3,2-*f*]quinoline-2,7,9-tricarboxylic Acid (3):
A mixture of **20** (100 mg, 0.269 mmol) and 0.1 M aq K_2CO_3 solution (10 mL) was stirred at 60°C for 10 h. The solution was acidified with 5 M HCl solution. The precipitate was collected by centrifugation, washed with water 2 times to give **3** as a brick-red solid (82 mg, 92%); mp > 300°C (dec).

IR (KBr): ν = 3436, 1717, 1684, 1653°C.

^1H NMR (DMSO-d_6): δ = 7.41 (s, 1H), 7.78 (s, 1H).

^{13}C NMR (DMSO-d_6): δ = 114.48, 124.94, 126.29, 126.55, 131.01, 132.07, 143.40, 143.96, 146.20, 161.13, 165.64, 168.92, 169.00, 179.14.

***N*-(3-methoxyphenyl)acetamide (22):**
A solution of *m*-anisidine **21** (97%, 120 g, 0.945 mol) in 2 M HCl containing a little ice was treated with a solution of NaOAc (600 g) in H_2O (3000 mL) followed by Ac_2O (420 mL). After shaking vigorously for 20 min, the mixture was cooled in an ice bath. The precipitate formed was collected by filtration and washed with H_2O to obtain **22** as a white solid (142.3 g, 91%); R_f 0.35 (5% MeOH in CH_2Cl_2). An analytical sample was obtained by recrystallization from aq EtOH; mp 80–81°C (Lit.[17] mp 81°C).

5′-Methoxy-2′-nitroacetanilide (23):
Method A: To a solution of **22** (4.99 g, 30.24 mmol) in Ac_2O (44 mL) maintained at 0–5°C was added a solution of HNO_3 (70%, 1.68 mL) in AcOH (5.4 mL). The mixture was stirred at 5°C for 6 h. Excess Ac_2O was carefully hydrolyzed by the addition of 3 M HCl (4.8 mL) and the mixture was diluted with water (200 mL). The precipitate formed was collected by filtration and washed well with water. The dry product was extracted in a Soxhlet with ligroin (bp 70–90°C) to give **23** as a yellow solid (3.62 g, 57%); mp 122–123°C (Lit.[18] mp 121–123°C); R_f 0.39 (CH_2Cl_2).

The ligroin insoluble residue was recrystallized from MeOH to obtain brown crystals of 3′-methoxy-4′-nitroacetanilide (1.31 g, 21%); mp 167–168°C (Lit.[19] mp 167°C). R_f 0.21 (CH_2Cl_2).

Method B:To a solution of **22** (77.5 g, 0.470 mol) in Ac_2O (680 mL) at r.t. was added a mixture of HNO_3 (70%, 26.1 mL) and AcOH (26 mL) and the temperature of the mixture was maintained at 40–45°C. The mixture was stirred at 25–40°C for an additional 6 h. Excess of Ac_2O was destroyed by the addition of 3 M HCl (78 mL). Water (2000 mL) was added and the precipitate formed was collected by filtration and washed with H_2O to obtain **23** as a yellow solid (61.94 g, 63%).

5-Methoxy-2-nitroaniline (10a):
A suspension of **23** (60 g, 0.286 mol) in concd HCl (700 mL) was refluxed for 5 h. After cooling to r.t. water (500 mL) was added. The precipitate was collected by filtration and recrystallized from MeOH to obtain yellow crystals (40.9 g, 85%); mp 128–129°C (Lit.[18] mp 126–128°C); R_f 0.48 (CH_2Cl_2).

Ethyl Pyruvate 5-Methoxy-2-nitrophenylhydrazone (24):
To a mixture of **10a** (34.6 g, 0.206 mmol) and 4 M HCl (250 mL, 1 mol) maintained at 0–5°C was slowly added a solution of $NaNO_2$ (17.04 g, 0.247 mol) in H_2O (162 mL). The mixture was stirred at 0–5°C for 1 h, after which the insoluble particles were removed by filtration at low temperature. The filtrate of diazonium salt solution was added into a mixture of ethyl 2-methylacetoacetate (90%, 33.0 g, 0.206 mol), EtOH (256 mL), KOH (67.1 g, 1.05 mol) and H_2O (350 mL) maintained at 2 ~ 5°C. The mixture was stirred at 5°C for 1 h and kept overnight at 5°C. The precipitate was collected by filtration and recrystallized from MeOH to afford brown crystals (35.77 g, 62%); mp 140–141°C.

IR (KBr): ν = 3280, 1717, 1615, 1231 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.40 (t, 3H, J = 7.11 Hz), 2.25 (s, 3H), 3.95 (s, 3H), 4.35 (q, 2H, J = 7.11 Hz), 6.52 (dd, 1H, J = 10.5, 3.0 Hz), 7.40 (d, 1H, J = 3.0 Hz), 8.16 (d, 1H, J = 10.5 Hz), 11.19 (br, 1H).

^{13}C NMR ($CDCl_3$): 11.78, 14.22, 55.93, 61.64, 97.93, 109.36, 126.92, 128.24, 139.48, 143.17, 164.46, 165.94.

Ethyl 4-Methoxy-7-nitroindole-2-carboxylate (25):
Polyphosphoric acid (265 g) was mixed carefully with **24** (30 g, 0.107 mol) and toluene (460 mL). The mixture was stirred at 100°C for 8.5 h. After cooling to r.t., the toluene layer was separated and the polyphosphoric acid layer was extracted with toluene (200 mL × 3). The combined toluene extracts were washed with aq $NaHCO_3$ solution and water, and dried (Na_2SO_4). Recrystallization from MeOH gave **25** as yellow crystals (6.60 g, 23%); mp 157–158°C; R_f 0.54 (CH_2Cl_2).

IR (KBr): ν = 3473, 1709, 1519, 1314, 1268 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.43 (t, 3H, J = 7.14 Hz), 4.08 (s, 3H), 4.44 (q, 2H, J = 7.14 Hz), 6.61 (d, 1H, J = 9.9 Hz), 7.42 (d, 1H, J = 2.7 Hz), 8.31 (d, 1H, J = 9.9 Hz), 10.33 (br, 1H).

^{13}C NMR ($CDCl_3$): δ = 14.34, 56.27, 61.41, 100.38, 107.30, 120.09, 125.78, 127.86, 128.31, 131.40, 160.71, 160.79.

Ethyl 7-amino-4-methoxyindole-2-carboxylate (7a):
A suspension of **25** (6.6 g, 25.0 mmol) in EtOH (350 mL) was hydrogenated over 10% Pd/C (0.8 g) at 150 psi for 12 h. Removal of the catalyst by filtration, followed by evaporation of solvent gave

7a (5.69 g, 97%), which was used in the following reaction without further purification; R_f 0.53 (5% MeOH in CH_2Cl_2).

6,8-Dimethoxycarbonyl-2-ethoxycarbonyl-4-methoxy-1H-pyrrole-[3,2-*h*]quinoline (26):
A mixture of **7a** (5.69 g, 24.3 mmol) and dimethyl *trans*-2-ketoglutaconate[14] (5.23 g, 30.4 mmol) in anhyd CH_2Cl_2 (500 mL) was stirred at r.t. for 12 h. Dry HCl gas was passed through the mixture for 10 h, and the mixture was stirred under O_2 overnight. The residue obtained after evaporation of solvent was washed with a small volume of EtOH, and recrystallized from EtOH to obtain **26** as light yellow crystals (7.2 g, 77%); mp 226–227°C; R_f 0.6 (5% MeOH in CH_2Cl_2).

IR (KBr): ν = 3458, 1711, 1493, 1231 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.46 (t, 3H), 4.07 (s, 3H), 4.10 (s, 3H), 4.12 (s, 3H), 4.46 (q, 2H), 7.45 (d, 1H, J = 2.6 Hz), 7.89 (s, 1H), 8.67 (s, 1H), 11.36 (b, 1H).

^{13}C NMR ($CDCl_3$): δ = 14.42, 52.71, 52.95, 55.76, 61.13, 93.90, 107.37, 120.58, 122.40, 127.72, 128.23, 132.65, 133.66, 136.56, 141.72, 157.51, 161.13, 165.39, 166.44.

6,8-Dimethoxycarbonyl-2-ethoxycarbonyl-1*H*-pyrrol[3,2-*h*]quinoline-4,5-dione (27):
To a solution of **26** (1.81 g, 4.69 mmol) in MeCN/H_2O (5:1, 1700 mL) was gradually added CAN (5.42 g, 28.14 mmol) at 0–2°C. The mixture was stirred for an additional 3.5 h at 0–2°C. Water (1000 mL) was added, and the mixture was extracted with EtOAc (3 × 250 mL). The combined extracts were washed with H_2O and dried (Na_2SO_4). The product was recrystallized from $CHCl_3$/hexane to obtain **27** as yellow crystals (1.42 g, 79%); mp 165–166°C (Lit.[15] mp 165°C); R_f 0.45 (5% MeOH in CH_2Cl_2).

4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[3,2-*h*]quinoline-2,6,8-tricarboxylic Acid (4):
A mixture of **27** (600 mg, 1.55 mmol) and 0.5 M aq K_2CO_3 solution (6 mL) was stirred at 60°C for 10 h. The solution was acidified with 5 M HCl. The precipitate was collected by centrifugation and washed two times with water to obtain **4** as a brick-red solid (465 mg, 91%); mp > 300°C (dec).

IR (KBr): ν = 3400, 1735, 1717, 1684 cm^{-1}.

^1H NMR (DMSO-d_6): δ = 7.05 (s, 1H), 7.79 (s, 1H).

^{13}C NMR (DMSO-d_6): δ = 113.45, 120.03, 123.02, 123.67, 129.43, 138.10, 147.01, 148.86, 161.47, 163.65, 167.80, 172.93, 178.83.

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