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Synthesis of Imidazolo Analogues of the Oxidation−Reduction Cofactor Pyrroloquinoline Quinone (PQQ)

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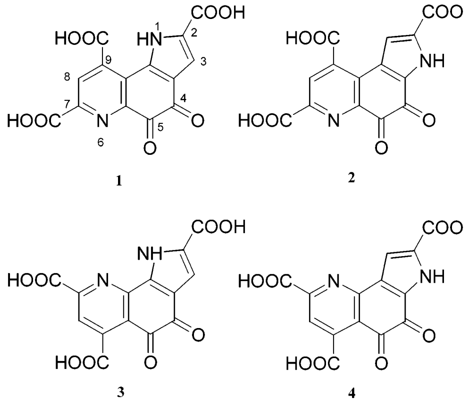
Abstract: Parallel syntheses of 2-hydro-, 2-methyl-, and 2-methoxycarbonylimidazo-7,9-dimethoxycarbonyl analogues of the oxidation−reduction cofactor pyrroloquinoline quinone [4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid] have been developed. The properties of the imidazolo analogues in relation to the corresponding pyrrole analogues will be important in assessing the origins of catalysis and biological activity in the cofactor, which has recently been shown to be a vitamin.

Pyrroloquinoline quinone (**1**, PQQ, methoxatin, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) is an oxidation−reduction cofactor found in methylotropic bacteria. The structure of the cofactor was elucidated in 1979, when it was isolated from *Psuedomonas*.[2] PQQ as well as other quinonoid cofactors have now been found in association with many enzymes from a variety of organisms.[3] In bacteria, in addition to being a redox cofactor, PQQ was shown to be a growth factor.[4] Important physiological roles have also been suggested for PQQ in eukaryotes.[5] Indeed, the documented role of PQQ as a micronutrient in mammals,[5] the absence of biosynthesis pathways for PQQ in eukaryotes, and the recent demonstration that PQQ is a required cofactor in a mammalian enzyme[6] have established PQQ as a vitamin.



1 2

3 4

FIGURE 1. Structure of PQQ and several isomers.

Preparation of key analogues of this vitamin will be important in understanding the mechanism of action of PQQ. Isomeric analogues of PQQ as well as analogues with simple isoelectronic substitutions may be particularly interesting in that regard. In addition, PQQ analogues, particularly those with altered redox potentials, may be usefully integrated into microsensors.[7] Previously, we prepared several PQQ isomers[8] (**2**−**4**, Figure 1), allowing the establishment of their properties and the basis for the determination of their presence or absence in representative biological systems.[9] We have synthesized and report here imidazolo analogues of PQQ, where the pyrrole ring is replaced by imidazole, to assess the importance of the pyrrole ring in the catalytic function of PQQ (**5**−**7**, Figure 2). As a mechanistically relevant equilibration of the tautomeric forms[10] of the imidazole ring during the course of catalysis may likely depend on the nature of the substituent at C-2, the syntheses have been developed to allow incorporation of variable functional groups in position 2.

In our plan for the synthesis of the targeted imidazole derivatives of pyrroloquinoline quinone (**5**−**7**), we started by assembling an appropriately substituted benzimidazole moiety and then constructed the quinoline ring system. This approach was analogous to the one taken by Corey and Tramontano in the synthesis of PQQ[11] and subsequently adapted by others to the synthesis of several isomeric[8] and nonisomeric analogues[12] of PQQ.

In the synthesis of the 2-methyl analogue **6** (Scheme 1) compound **8** was prepared by directly nitrating commercially available 2-methoxy-4-nitroaniline followed by selective reduction of the *o*-nitro group using conditions analogous to those reported to be favorable to the reduction of nitro groups ortho to amino and hydroxy

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[10] Itoh, S.; Fukui, Y.; Haranou, S.; Ogino, M.; Komatsu, M.; Oshiro, Y. *J. Org. Chem.* **1992**, *57*, 4452. Only a single tautomeric form of each of the final imidazole analogues **5**−**7** is shown for each isomer. While the spectroscopic properties are consistent with the presence of a single tautomeric form, we cannot specify which form is present. In Figure 2 and in Schemes 1−3, only a single tautomeric form of the imidazole is shown by convention.

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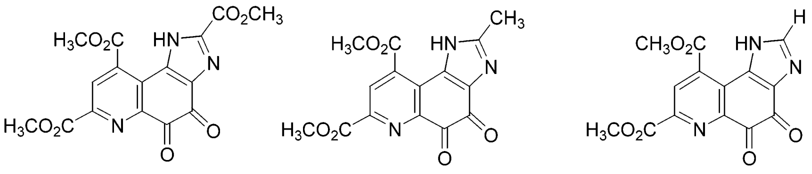
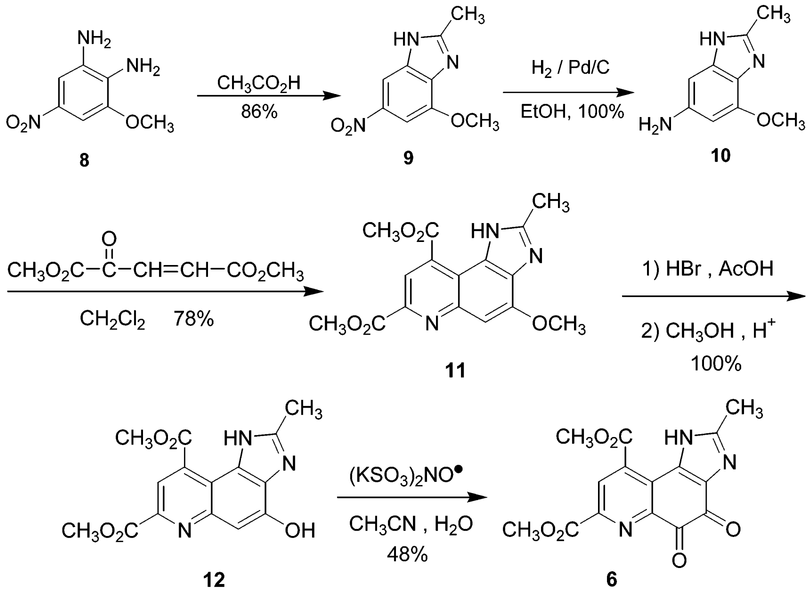


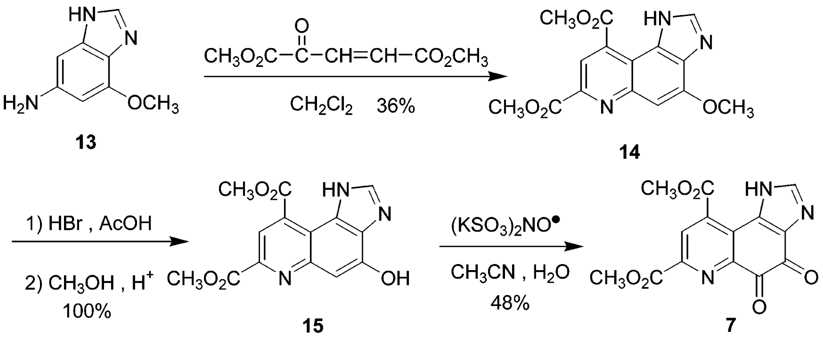
5 6 7

FIGURE 2. Structures of imidazole analogues of PQQ.

SCHEME 1



SCHEME 2



substituents.[13] Compound **8** was previously synthesized in three steps beginning with 3,5-dinitroanisole.[14]

The treatment of 2-amino-3-methoxy-5-nitroaniline **8** with glacial acetic acid produced the nitrobenzimidazole **9**, which was subsequently reduced by catalytic hydrogenation to yield the amine **10**. Coupling of **10** with dimethyl *trans*-2-ketoglutaconate under Doebner–von Miller conditions[11] resulted in the imidazoquinoline **11** in good yield. Demethylation of **11** was carried out in quantitative yield using HBr in glacial acetic acid. During this process, the two ester groups were also cleaved and re-esterification was acomplished quantitatively without intermediate purification to yield **12**. This product was then treated with Fremy's salt in aqueous acetonitrile to generate the target **6**.

In the synthesis of the 2-hydroimidazolo-PQQ analogue **7** (Scheme 2), we employed the known benzimidazole **13**,[15] which was prepared from compound **8** and subsequently treated with formic acid in the presence of palladium on carbon followed by subsequent base hydrolysis of the intermediate formanilide to generate the amine **13**. Compound **13** also has been previously synthesized by an alternative five-step method.[14] When treated with dimethyl *trans*-2-ketoglutaconate, **13** underwent cyclization to form the quinoline derivative **14** in modest yield. Removal of the methoxy group of **14** was accomplished quantitatively using HBr in glacial acetic acid, followed by quantitative re-esterification of the two cleaved carboxylic acid groups to yield **15**. Oxidation of **15** using Fremy's salt led to the quinone **7**.

The synthesis of the imidazolo-PQQ analogue **5** (Scheme

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(13) Terpko, M. O.; Heck, R. F. *J. Org. Chem.* **1980**, *45*, 4992.

(14) Gillespie, H. B.; Engelman, M.; Graff, S. *J. Am. Chem. Soc.* **1954**, *76*, 3531

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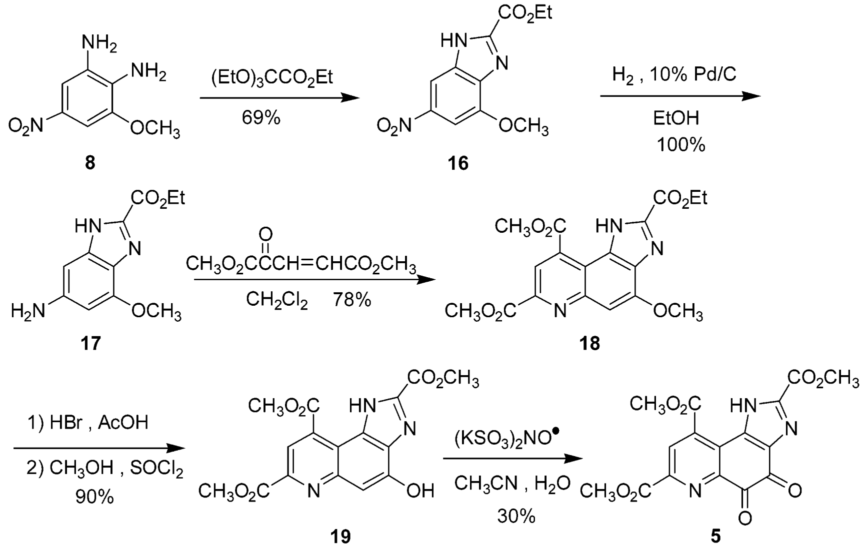
SCHEME 3



3) proved initially to be quite challenging. Several approaches were undertaken. Alternative routes to compound **5** were considered starting from **11** and **14**. We attempted without success to selectively oxidize the 2-methyl group in **11**.[16] A lithiation–carboxylation sequence at the 2-position of **14** was also attempted without success.[17] Despite the use of a variety of protecting conditions such as carbon dioxide,[18] pyrrolidine methylation,[19] and formaldehyde,[20] the benzimidazole nitrogen could not be protected effectively, rendering lithiation at C-2 impractical.

Compound **5** was finally synthesized starting from the diamine **8** using procedures analogous to those employed for the previous two analogues (**6** and **7**). However, the initial formation of the key benzimidazole required a special condensing agent. The benzimidazole **16** was obtained in good yield by condensation of **8** with ethyl triethoxyacetate.[21] The nitrobenzimidazole **16** was quantitatively reduced to the amine **17** by palladium-catalyzed hydrogenation, and the pyridine ring was assembled using Doebner–von Miller conditions to yield **18**. Deprotection of methoxy in **18** was achieved by prolonged treatment in HBr in acetic acid with simultaneous hydrolysis of all three ester groups. Re-esterification without purification led to the phenolic trimethyl ester **19** in excellent overall yield. Oxidation of **19** with Fremy's salt led to the quinone **5** in modest yield.

In summary, several imidazolo analogues of pyrroloquinoline quinone were synthesized to further investigate the importance of the pyrrole ring in the catalytic reactions of the native coenzyme. Substituting an imidazole ring for the pyrrole ring of PQQ should have interesting effects on the catalytic power of these quinones, and we are expecting these molecules to have improved catalytic potential over the corresponding PQQ analogues **2**–**4**. The catalytic properties of analogues **5**–**7** are currently under investigation and will be reported elsewhere. The ester analogues (**5**–**7**) have been targeted as final products rather than the carboxylic acids, as they are more conveniently used in the analysis of the model catalytic properties of the cofactor analogues. The demethylated forms of **5**–**7** may be useful eventually in studies with reconstituted enzymes and in studies of vitamin function.

Experimental Section

2-Ethoxycarbonyl-4-methoxy-6-nitrobenzimidazole (16). A mixture of compound **8** (500 mg, 2.73 mmol) and ethyl triethoxyacetate (3.60 g, 16.4 mmol) was heated under nitrogen at 100 °C for 18 h producing a paste too thick to be stirred. The reaction mixture was then cooled to room temperature and washed with diethyl ether to give a yellowish powder (500 mg, 1.89 mmol, 69%). An analytical sample was recrystalized from methylene chloride–acetone to produce a pale yellow solid: mp 298–299 °C; ^1H NMR (DMSO-d_6 δ) 1.38 (t, 3H, $J = 3.2$ Hz), 3.99 (s, 3H), 4.41 (q, 2H, $J = 3.2$ Hz), 7.20 (d, 1H, $J = 2$ Hz), 7.90 (d, 1H, $J = 2$ Hz), 12.34 (br, 1H); ^{13}C NMR (DMSO-d_6 δ) 13.86, 56.72, 63.27, 102.46, 114.02, 125.92, 129.66, 142.40, 146.05, 150.23, 156.41; TLC $R_f = 0.50$ (5% CH_3OH in CH_2Cl_2). Anal. Calcd for $C_{11}H_{11}N_3O_5$: C, 49.81; H, 4.18; N, 15.84. Found: C, 50.07; H, 4.26; N, 15.71.

2-Ethoxycarbonyl-6-amino-4-methoxybenzimidazole (17). Compound **16** (300 mg, 1.13 mmol) was suspended in absolute ethanol (50 mL). To the mixture was added 10% Pd/C (54 mg). The mixture was stirred at room temperature under 80 psi of hydrogen for 4 h. The catalyst was removed by filtration and rinsed with ethanol. The ethanol was then removed in vacuo to yield a light brown powder (285 mg, 1.13 mmol, 100%): mp 233–236 °C; ^1H NMR (CD_3OD δ) 1.43 (t, 3H, $J = 3.2$ Hz), 3.90 (s, 3H), 4.41 (q, 2H, $J = 3.2$ Hz), 6.46 (d, 1H, $J = 2$ Hz), 6.48 (d, 1H, $J = 2$ Hz); ^{13}C NMR (CD_3OD δ) 14.60, 56.64, 64.36, 99.52, 104.21, 113.10, 134.11, 146.18, 148.39, 152.10, 156.44; TLC $R_f = 0.65$ (10% CH_3OH in CH_2Cl_2). Anal. Calcd for $C_{11}H_{13}N_3O_3 \cdot 0.5H_2O$: C, 54.08; H, 5.79; N, 17.19. Found: C, 54.28; H, 5.89; N, 17.20.

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2-Ethoxycarbonyl-4-methoxy-7,9-dimethoxycarbonyl-1*H*-imidazolo[5,4-f]quinoline (18). A mixture of compound **17** (90 mg, 0.383 mmol), dimethyl *trans*-2-ketoglutaconate (91 mg, 0.528 mmol), and dry methylene chloride (10 mL) was stirred under nitrogen at room temperature for 90 h. Methylene chloride (40 mL) was added, and dry hydrogen chloride was bubbled through the solution for 3 h. Oxygen and dry hydrogen chloride were then bubbled through the solution for 4 h. The solvent was removed in vacuo, and the resulting solid was dissolved in methylene chloride (40 mL). The methylene chloride solution was washed with 0.2 M sodium bicarbonate and with brine. The methylene chloride extract was dried over anhydrous sodium sulfate, evaporated to dryness, and subsequently recrystallized from methylene chloride−hexane to yield a pale yellow powder (115 mg, 0.297 mmol, 78%): mp > 300 °C; ^{1}H NMR (DMSO-d_6 δ) 1.42 (t, 3H, $J = 7.2$ Hz), 3.96 (s, 3H), 3.97 (s, 3H), 4.09 (s, 3H), 4.37 (q, 2H, $J = 7.2$ Hz), 7.65 (s, 1H), 8.02 (s, 1H), 12.52 (br, 1H); ^{13}C NMR (DMSO-d_6 δ) 13.97, 52.59, 52.63, 56.65, 63.41, 106.18, 116.40, 117.98, 122.30, 122.87, 138.16, 145.61, 145.83, 149.96, 150.19, 154.96, 164.62, 168.21; TLC $R_f = 0.48$ (5% CH_3OH in CH_2Cl_2). Anal. Calcd for $C_{18}H_{17}N_3O_7$: C, 55.81; H, 4.42; N, 10.85. Found: C, 55.52; H, 4.56; N, 10.72.

4-Hydroxy-2,7,9-trimethoxycarbonyl-1*H*-imidazolo[5,4-f]quinoline (19). A mixture of compound **18** (500 mg, 1.29 mmol) and 33% HBr in glacial acetic acid (50 mL) was placed in a round-bottom flask equipped with a condenser and an argon-filled balloon. The mixture was heated under vigorous reflux for 5 days. Each day, the acid mixture was removed under vacuum and replaced with 33% HBr in glacial acetic acid (50 mL), and a new argon-filled balloon was applied. The solvent was then removed in vacuo. Dry methanol (80 mL) and thionyl chloride (1 mL) were added to the flask. The resulting solution was refluxed under nitrogen overnight. The solvent was removed in vacuo to yield a brown powder (417 mg, 1.90 mmol, 90%). An analytical sample was recrystalized from methanol−methylene chloride−hexane to give a yellow powder: mp > 300 °C; ^{1}H NMR (CD_3OD δ) 4.11 (s, 3H), 4.14 (s, 3H), 4.16 (s, 3H), 7.68 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (DMSO-d_6 δ) 53.81, 54.38, 54.58, 56.48, 118.18, 118.94, 119.52, 119.67, 125.06, 125.15, 125.26, 142.41, 144.23, 146.63, 152.48, 154.27, 157.76, 157.90, 163.20, 170.18; TLC $R_f = 0.06$ (5% CH_3OH in CH_2Cl_2). HRMS-FAB (*m*/*z*): $[M + Na]^+$ calcd for $C_{16}H_{13}N_3O_7H$ 360.0831; found 360.0806.

2,7,9-Trimethoxycarbonyl-1*H*-imidazolo[5,4-f]quinoline-4,5-dione (5). A mixture of compound **19** (100 mg, 0.332 mmol), KH_2PO_4 (70 mg), and K_2HPO_4 (100 mg) was stirred in 5:1 acetonitrile−water (60 mL) for 1 h. The pH was adjusted to 7 by addition of dilute aqueous HCl. A solution of Fremy's salt (178 mg, 0.664 mmol) and K_2HPO_4 (67 mg) in water (7 mL) was added in fractions, and the mixture was stirred at room temperature for 12 h. The solution was brought to pH 7 by addition of dilute NaOH. A solution of Fremy's salt (150 mg, 0.559 mmol) and K_2HPO_4 (67 mg) in water (7 mL) was added in fractions. The mixture was stirred at room temperature for an additional 24 h. The mixture was extracted with methylene chloride, and the combined organic fractions were dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was recrystallized from methylene chloride−hexane to yield an orange powder (37 mg, 30%): mp 290−293 °C; ^{1}H NMR (CD_3OD δ) 3.99 (s, 3H), 4.03 (s, 3H), 4.08 (s, 3H), 8.13 (s, 1H); ^{13}C NMR (DMSO-d_6 δ) 52.83, 52.88, 55.67, 120.04, 126.59, 129.84, 139.22, 145.92, 146.72, 149.41, 163.82, 167.09, 171.31, 173.77; TLC $R_f = 0.42$ (5% CH_3OH in CH_2Cl_2). HRMS-FAB (*m*/*z*): $[M + Na]^+$ calcd for $C_{16}H_{11}N_3O_8Na$ 396.0444; found 396.0439.

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Supporting Information Available: Experimental details are given for general procedures and for the preparation of the imidazolo analogues **6** and **7** as well as for the synthetic intermediates described in the preparation of compounds **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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