1, H-4'), 4.66 (dd, 1, H-2', $J_{1',2'}$ = 4.4 Hz, $J_{2',3'}$ = 6.4 Hz), 4.88 (dd, 1, H-3', $J_{3',4'}$ = 3.0 Hz), 5.06 (d, 1, H-1'), 6.27 (d, 1, H-4, $J_{3,4}$ = 4.0 Hz), 6.94 (d, 1, H-3), 9.43 (s, 1, CHO), 10.74 (br, 1, NH).

3,4-Dihydro-6-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-pyrrolo[1,2-*a*]pyrazine (16): colorless foam, 75%; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3, CH_3), 1.59 (s, 3, CH_3), 3.68 (dd, 1, H-5'a, $J_{4',5'a}$ = 4.0 Hz, $J_{5'a,5'b}$ = 12.1 Hz), 3.80 (dd, 1, H-5'b), 3.89–3.97 (m, 4, H-3, H-4), 4.18 (q, 1, H-4'), 4.85 (d, 1, H-1', $J_{1',2'}$ = 5.3 Hz), 4.79, 4.89 (each t, 1 each, H-2', H-3'), 6.22, 6.37 (each d, 1 each, H-7, H-8, $J_{1,2}$ = 4.0 Hz), 8.13 (apparent s, 1, H-1).

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)pyrrolo[1,2-*a*]quinoxaline (20): colorless foam, 81%; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3, CH_3), 1.61 (s, 3, CH_3), 3.78 (dd, 1, H-5', $J_{4',5'a}$ = 3.4 Hz, $J_{5'a,5'b}$ = 12.1 Hz), 3.91 (dd, 1, H-5'b, $J_{4',5'b}$ = 3.4 Hz), 4.41 (q, 1, H-4'), 4.93 (dd, 1, H-3', $J_{2',3'}$ = 5.7 Hz, $J_{3',4'}$ = 3.4 Hz), 5.17 (t, 1, H-2'), 5.50 (d, 1, H-1'), 6.90, 7.03 (each d, 2 each, H-2, H-3, J = 4.4 Hz), 7.45–7.58 (m, 2, H-7, H-8), 7.98 (d, 1, H-9, J = 8.0 Hz), 8.40 (d, 1, H-6, J = 8.4 Hz).

2-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)quinoxaline (22): colorless foam, 87%; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3, CH_3), 1.68 (s, 3, CH_3), 3.69–3.80 (br, 1, H-5'a), 4.04 (dd, 1, H-5'b, $J_{4',5'b}$ = 2.4 Hz, $J_{5'a,5'b}$ = 12.4 Hz), 4.57 (q, 1, H-4'), 4.95–5.03 (m, 2, H-2', H-3'), 5.22 (br, 1, OH), 5.38 (d, 1, H-1', $J_{1',2'}$ = 3.7 Hz), 7.26–7.85 (m, 2, H-6, H-7), 8.05–8.18 (m, 2, H-5, H-8), 8.91 (s, 1, H-3).

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Registry No. **3**, 108007-57-4; **4**, 108007-58-5; **5**, 112969-20-7; **6**, 113033-67-3; **7** (diastereomer 1), 112969-21-8; **7** (diastereomer 2), 113033-68-4; **8** (diastereomer 1), 113033-69-5; **8** (diastereomer 2), 113033-70-8; **11a**, 112969-22-9; **11b**, 112969-23-0; **11c**, 112969-24-1; **12a**, 112969-28-5; **12b**, 112969-29-6; **12c**, 112969-30-9; **13**, 112969-33-2; **14**, 112969-25-2; **15**, 112969-31-0; **16**, 112969-34-3; **17**, 112969-26-3; **18**, 112969-27-4; **19**, 112987-81-2; **20**, 112987-82-3; **21**, 112969-32-1; **22**, 112969-35-4; $MeNH_2$, 74-89-5; $PhNH_2$, 62-53-3; $H_2NCH_2CH_2NH_2$, 107-15-3; *o*-$H_2NC_6H_4NH_2$, 95-54-5.

Total Synthesis of Prodigiosin, Prodigiosene, and Desmethoxyprodigiosin: Diels–Alder Reactions of Heterocyclic Azadienes and Development of an Effective Palladium(II)-Promoted 2,2'-Bipyrrole Coupling Procedure

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The total synthesis of prodigiosin (1), a red pigment first isolated from *Serratia marcescens*, possessing the characteristic pyrrolylpyrromethene skeleton of a class of naturally occurring polypyrroles exhibiting antimicrobial and cytotoxic properties, is detailed. The approach is based on the application of an inverse electron demand Diels–Alder reaction of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate in a 1,2,4,5-tetrazine → 1,2-diazine → pyrrole strategy for preparation of prodigiosin pyrrole ring B and the subsequent implementation of an effective intramolecular palladium(II)-promoted 2,2'-diaryl coupling for construction of the prodigiosin 2,2'-bipyrrole AB ring system. In situ generated activated ester derivatives of pyrrole-1-carboxylic acid or the use of pyrrole-1-carboxylic acid anhydride proved suitable for the generation of *mixed* 1,1'-carbonyldipyrrole compounds for use in the palladium(II)-promoted mixed, 2,2'-bipyrrole coupling. Extensions of this approach to the preparation of the naturally occurring parent pyrrolylpyrromethene, prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosene (**2e**, desmethoxyprodigiosin) are detailed. A comparison of the in vitro cytotoxic properties of prodigiosin (1), prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosene (**2e**) are reported and reveal exceptional cytotoxic potency (3.7×10^{-4} μg/mL ≡ 3.7×10^{-10} g/mL) for prodigiosin against 9PS (P388) mouse leukemia which may be attributed to the presence of the prodigiosin C-6 methoxy substituent.

Prodigiosin (1), a red pigment first isolated from *Serratia marcescens*,[1] was the initial member of a class of naturally occurring polypyrroles possessing a common, characteristic pyrrolylpyrromethene skeleton[2] which now include prodigiosene (**2a**), norprodigiosin (**2b**), undecylprodigiosin (**2c**), nonylprodigiosin (**2d**), cyclic nonylprodigiosin (**3**), cycloprodigiosin (**4**), metacycloprodigiosin (**5**), and **6**, which have been shown to possess potent antimicrobial and cytotoxic properties.[3] Extensive past efforts utilized in the preparation and structural confirmation of the naturally occurring[1,4] and synthetic[5] pro-

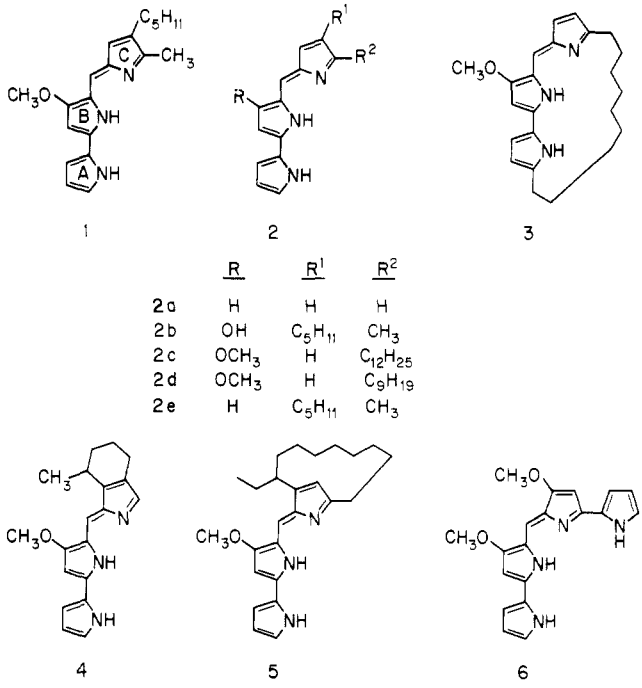
(1) Prodigiosin (methyl pentylprodigiosin, 1). (a) Isolation and characterization: Wrede, F.; Rothhass, A. *Z. Physiol. Chem.* **1934**, *226*, 95 and references cited therein. (b) Biosynthesis: Wasserman, H. H.; McKeon, J. E.; Santer, U. V. *Biochem. Biophys. Res. Commun.* **1960**, *3*, 146. Santer, U. V.; Vogel, H. J. *Fed. Proc.* **1956**, *15*, 345; *Biochem. Biophys. Acta* **1956**, *19*, 578. Structure determination (total synthesis, partial synthesis): (c) Wasserman, H. H.; McKeon, J. E.; Smith, L.; Forgione, P. *J. Am. Chem. Soc.* **1960**, *82*, 506; *Tetrahedron Suppl.* **1966**, *8* (Part II), 647. (d) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635; **1960**, *82*, 5510. (e) Castro, A. J.; Deck, J. F.; Hugo, M. T.; Lowe. E. J.; Marsh, J. P., Jr.; Pfeiffer, B. J. *J. Org. Chem.* **1963**, *28*, 857. Castro, A. J.; Corwin, A. H.; Waxham, F. J.; Beilby, A. L. *J. Org. Chem.* **1959**, *24*, 455. (f) Review: Jackson, A. H.; Smith, K. M. *Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley-Interscience: New York, (i) 1973, (ii) 1984; (i) Vol. 1, pp 227–232, (ii) Vol. 6, pp. 258.

(2) (a) Review: Gerber, N. N. *Crit. Rev. Microbiol.* **1974**, *3*, 469. (b) Prodigiosene (isolation): Ahn, T. S.; Choi, Y. K.; Hong, S. W. *Misaengmul Hakhoe Chi* **1977**, *15*, 159.

(3) Thompson, P. E.; McCarty, D. A.; Bayles, A.; Reinertson, J. W.; Cook, A. R. *Antibiot. Chemother.* **1956**, *6*, 337. Williams, R. P.; Hearn, W. R. *Antibiotics. Biosynthesis*; Gottlieb, D., Shaw, P. D., Eds.; Springer-Verlag: Berlin, Germany, 1967; Vol. II, p 410. Castro, A. J. *Nature (London)* **1967**, *213*, 903. Ballester, M.; Ballester, J. M.; Belaich, J. P. *Microbiol. Ecol.* **1977**, *3*, 289. Matsunaga, S.; Fusetani, N.; Hashimoto, K. *Experientia* **1986**, *42*, 84. Alonzo, V. *Ig. Mod.* **1984**, *81*, 557. Immunostimulate: Sobolev, V. R.; Vaisberg, G. E. *Khim.-Farm. Zh.* **1977**, *11*, 142; Zhang, G.; Feiging, L.; Bingyuan, W.; Huizhen, G.; Jingbo, L.; Shibing, G.; Xiangguang, L. *Shanghai Diyi Yixueyuan Xuebao* **1981**, *8*, 401. Cytotoxic activity: Barbagallo, C.; Luciana, M. T.; Pietro, P.; Carmen, M. T. *Riv. Biol. Norm. Patol.* **1979**, *5*, 25. See also: Jpn. Pat. 80 162 768, 1980; *Chem. Abstr.* **1981**, *94*, 114722h.

(4) Hearn, W. R.; Elson, M. K.; William, R. H.; Medina-Castro, J. *J. Org. Chem.* **1970**, *35*, 142.

0022-3263/88/1953-1405$01.50/0

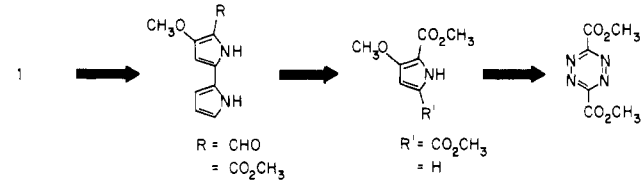


	R	R^1	R^2
2a	H	H	H
2b	OH	C_5H_{11}	CH_3
2c	OCH_3	H	$C_{12}H_{25}$
2d	OCH_3	H	C_9H_{19}
2e	H	C_5H_{11}	CH_3

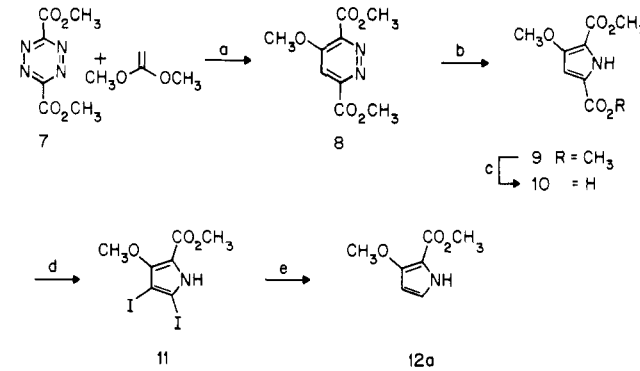
digiosenes have relied on conventional methods for monopyrrole preparation and the subsequent use of indirect approaches to electrophilic, intermolecular 2,2′-bipyrrole coupling[1c,d,6] for assemblage of the prodigiosin AB ring system.[7]

Herein we describe the development of a complementary approach to the construction of the pyrrolylpyrromethene skeleton of the prodigiosenes which has resulted in the total syntheses of prodigiosin (**1**),[7] prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosene [desmethoxyprodigiosin (**2e**)].[1] The approach is based on the application of an inverse electron demand Diels–Alder reaction[8] of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate in the implementation of a 1,2,4,5-tetrazine → 1,2-diazine → pyrrole strategy[9,10] for preparation of methyl 3-methoxypyrrole-2-carboxylate constituting prodigiosin ring B (Scheme I). A subsequent palladium(II)-promoted, intramolecular 2,2′-bipyrrole coupling reaction proved suited for construction of the

Scheme I



Scheme II[a]



[a] (a) Dioxane, 25 °C, 94%; (b) Zn, CH_3CO_2H, 25 °C, 68%; (c) LiOH, THF–CH_3OH–H_2O (3:1:1), 25 °C, 91%; (d) NaI, I_2, $NaHCO_3$, $ClCH_2CH_2Cl$–H_2O (1:1), 25 °C, 89%; (e) 5% Pd/C (catalyst), H_2 (1 atm), K_2CO_3, CH_3OH, 25 °C, 96%.

prodigiosin 2,2′-bipyrrole AB ring system and provides a general solution to the preparation of *mixed*, electron-deficient 2,2′-bipyrroles.[7] The subsequent application of this methodology in the total syntheses of the naturally occurring parent pyrrolylpyrromethene, prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosene (**2e**, desmethoxyprodigiosin) required for comparative determination of the structural features responsible for or potentiating the antimicrobial and cytotoxic properties of the prodigiosenes is detailed.

Prodigiosin Ring B Preparation: Implementation of the 1,2,4,5-Tetrazine → 1,2-Diazine → Pyrrole Diels–Alder Strategy. Treatment of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate[11] (**7**) with 1,1-dimethoxyethylene[12] provided the Diels–Alder cycloadduct **8** (25 °C, 3 h, dioxane, 94%)[9] (Scheme II). Reductive ring contraction of the electron-deficient 1,2-diazine **8** (10 equiv of zinc, HOAc, 25 °C) provided dimethyl 3-methoxypyrrole-2,5-dicarboxylate (**9**, 68%),[9] thus establishing the prodigiosin monopyrrole B ring. Effective differentiation of the C-2/C-5 carboxylates of **9** by selective hydrolysis [1.0 equiv of LiOH, THF–H_2O–CH_3OH (3:1:1), 25 °C, 78 h, 91%] of the electronically and sterically more accessible C-5 methoxycarbonyl group *cleanly* provided 3-methoxy-2-(methoxycarbonyl)pyrrole-5-carboxylic acid (**10**). Attempts to accelerate the rate of selective ester hydrolysis employing excess base (1.5–2.0 equiv of LiOH) or more vigorous reaction conditions (45–85 °C) resulted in competitive, subsequent C-2 methoxycarbonyl hydrolysis and provided 3-methoxypyrrole-2,5-dicarboxylic acid. The product of initial C-2 methoxycarbonyl hydrolysis, 3-methoxy-5-(methoxycarbonyl)pyrrole-2-carboxylic acid,

(5) Synthetic prodigiosenes: (a) Brown, D.; Griffiths, D.; Rider, M. E.; Smith, R. C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 455. (b) Berner, H.; Schultz, G.; Reinschagen *Monatsh. Chem.* **1978**, *109*, 137; **1977**, *108*, 2259. Champaigne, E.; Shutske, G. M. *J. Heterocycl. Chem.* **1976**, *13*, 497. Hearn, W. R.; Elson, M. K.; Williams, R. H.; Medina-Castro, J. *J. Org. Chem.* **1970**, *35*, 142. Castro, A. J.; Gale, G. R.; Means, G. E.; Tertzakran, G. *J. Med. Chem.* **1967**, *10*, 29 (antimicrobial properties). Ermili, A.; Castro, A. J. *J. Heterocycl. Chem.* **1966**, *3*, 521. Dolphin, D.; Harris, R. L. N.; Huppatz, J. L.; Johnson, A. W.; Kay, I. T.; Leng, J. *J. Chem. Soc. C* **1966**, *98*. Mukherjee, P. P.; Goldschmidt, M. E.; Williams, R. P. *Biochem. Biophys. Acta* **1967**, *136*, 182. Williams, R. P.; Goldschmidt, M. E.; Gott, C. L. *Biochem. Biophys. Res. Commun.* **1965**, *19*, 177. Kresze, G.; Morper, M.; Bijev, A. *Tetrahedron Lett.* **1977**, 2259. Broadhurst, M. J.; Grigg, R.; Johnson, A. W. *J. Chem. Soc., Perkin Trans. 1*, **1972**, 2111.

(6) Jones, R. A.; Bean, G. P. *The Chemistry of Pyrroles*; Academic: New York, 1977. Bordner, J.; Rapoport, H. *J. Org. Chem.* **1965**, *30*, 3824; **1964**, *29*, 2727. Castagnoli, N., Jr.; Rapoport, H. *J. Am. Chem. Soc.* **1962**, *84*, 2178. Rapoport, H.; Castagnoli, N., Jr.; Holden, K. G. *J. Org. Chem.* **1964**, *29*, 883. Grigg, R.; Johnson, A. W.; Wasky, J. W. F. *J. Chem. Soc.* **1983**, 359. Grigg, R.; Johnson, A. W. *Ibid.* **1964**, 3315. Bullock, E.; Grigg, R.; Johnson, A. W.; Wasley, J. W. F. *Ibid.* **1963**, 2336. For a recent, alternative approach, see: Hinz, W.; Jones, R. A.; Patel, S. U.; Karatza, M. H. *Tetrahedron* **1986**, *42*, 3753.

(7) Boger, D. L.; Patel, M. *Tetrahedron Lett.* **1987**, *28*, 2499.

(8) Boger, D. L. *Chem. Rev.* **1986**, *86*, 781. Boger, D. L. *Tetrahedron* **1983**, *39*, 2869.

(9) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Yohannes, D. *J. Org. Chem.* **1984**, *49*, 4405.

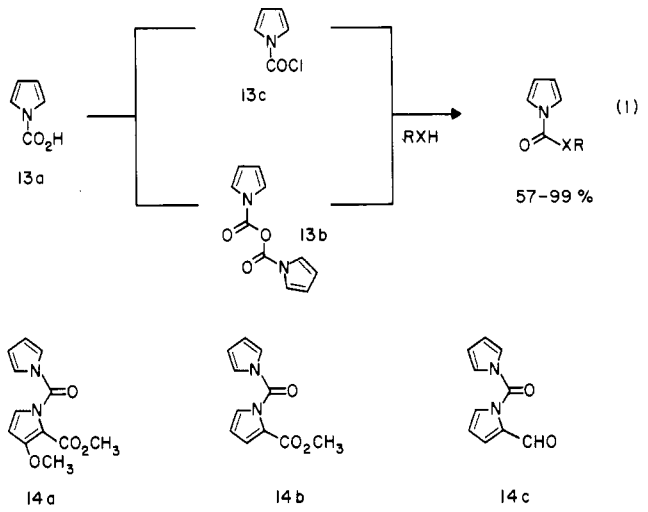
(10) Bach, N. J.; Kornfeld, E. C.; Jones, N. D.; Chaney, M. O.; Dorman, D. E.; Paschall, Clemens, J. A.; Smalstig, E. B. *J. Med. Chem.* **1980**, *23*, 481. Gewald, K.; Hain, U. *Synthesis* **1984**, *1*, 62.

(11) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Huber, F. X.; Sauer, J. *J. Org. Chem.* **1985**, *50*, 5377.

(12) 1,1-Dimethoxyethylene, 1-heptyn-3-ol, 3-butyn-1-ol, 4-pentyn-1-ol, 2-pentyn-1-ol, and 4-penten-1-ol are commercially available from Wiley Organics. 1-Heptyn-3-one was prepared by treatment of 1-heptyn-3-ol with Jones reagent [2.0 equiv, CH_2Cl_2–H_2O (1:1)]. 3-Butyn-1-ol and 2-pentyn-1-ol were converted to *tert*-butyldimethylsilyl ethers by the procedure described: Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

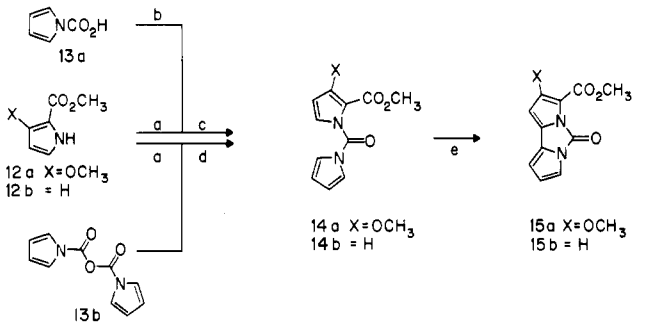
was not detected. Room temperature iodinative decarboxylation[13] of **10** and subsequent hydrogenolysis[14] of the resulting pyrrole diiodide **11** provided methyl 3-methoxypyrrole-2-carboxylate (**12a**). Direct thermal decarboxylation of **10** to provide **12a** proved unsuccessful.[15,16]

Preparation of the Prodigiosin and Prodigiosene AB Ring System: Development of an Effective, Intramolecular Palladium(II)-Promoted 2,2′-Bipyrrole Coupling Reaction. Implementation of an intramolecular, 2,2′-bipyrrole coupling reaction required a direct, controlled, and selective method for the preparation of mixed, electron-deficient 1,1′-carbonyldipyrrole compounds. Typically, the preparation of pyrrole *N*-carbonyl compounds is based on the nucleophilic coupling of 1,1′-carbonyldipyrrole (*N,N′*-carbonyldipyrrole) or the selective N-acylation of pyrrole with readily available, accessible acylating agents. In the instances when the nucleophilic displacement reaction of 1,1′-carbonyldipyrrole would be expected to be nonselective or unreactive with systems for which no activated acylation reagent is available, e.g., **12a,b**, the pyrrole *N*-carbonyl derivatives were inaccessible. We have detailed the investigation of several methods of carboxylate activation of pyrrole-1-carboxylic acid (**13a**),[18] the preparation and characterization of pyrrole-1-carboxylic anhydride (**13b**),[17a] and their use in selective, controlled coupling reactions with representative nucleophiles including electron-deficient pyrroles leading to the preparation of the corresponding pyrrole *N*-carbonyl compounds (eq 1).[17]



Consistent with observations made in this investigation, treatment of pyrrole-1-carboxylic acid (**13a**)[18] with triphenylphosphine–carbon tetrachloride[19] followed by the addition of the sodium salt of pyrrole **12a** or treatment of pyrrole-1-carboxylic acid anhydride (**13b**)[17] with the sodium salt of pyrrole **12a** provided the mixed 1,1′-carbonyldipyrrole derivative **14a** (Scheme III). Similarly, activation of pyrrole-1-carboxylic acid (**13a**) under a variety of conditions [**13a**, $(COCl)_2$, catalytic DMF or **13a**, $Me_2C{=}C(Cl)NMe_2$ (in situ acid chloride formation);[17,18b] **13a**, DCC; **13a**, Ph_3P, CCl_4, CH_3CN, 25 °C, 3 h[17] (in situ pyrrole-1-carboxylic anhydride formation)] followed by the addition of the sodium salt of **12b** or treatment of pyrrole-1-carboxylic anhydride (**13b**)[17] with the sodium salt of **12b** provided the mixed, electron-deficient 1,1′-carbonyldipyrrole **14b**. Intramolecular palladium(II)-promoted[20] 2,2′-bipyrrole coupling of **14a,b** provided **15a,b** and was found to be most effectively conducted by using stoichiometric, polymer-supported palladium(II) acetate (2–3% Pd, 1% cross-linked polystyrene).[21] The use of

Scheme III[a]



[a] (a) NaH, THF; (b) **13a**, $(COCl)_2$, catalytic DMF, THF, or **13a**, DCC, CH_2Cl_2; (c) THF, 25 °C, **14b** (89%, 67% respectively); (d) **13b**, THF; **14b** (69%), **14a** (52%); (e) see Table I: Ⓟ–$Pd(OAc)_2$, CH_3COOH, 80 °C, 12 h, 96% **15a**; 36 h, 90% **15b**.

Table I. Palladium(II)-Promoted Intramolecular 2,2′-Bipyrrole Formation

substrate	conditions	product	yield,[a,b] %
14a	CH_3COOH, 80 °C, 12 h, 0.34 equiv of $Pd(OAc)_2$	**15a**	34 (71)
	CF_3COOH, 25 °C, 48 h, 2.0 equiv of $Pd(OAc)_2$		0 (0)
	CH_3COOH, 80 °C, 12 h, Ⓟ–$Pd(OAc)_2$[c]		96 (96)
14b	CH_3COOH, 80 °C, 12 h, 0.34 equiv of $Pd(OAc)_2$	**15b**	36 (64)
	CF_3COOH, 25 °C, 48 h, 2.0 equiv of $Pd(OAc)_2$		42 (55)
	CH_3COOH, 80 °C, 36 h, Ⓟ–$Pd(OAc)_2$[d]		90 (90)
14c[e]	CH_3COOH, 80 °C, 12 h, Ⓟ–$Pd(OAc)_2$[d]	**15c**	0 (0)[e]
	CH_3CN, 80 °C, 12 h, Ⓟ–$Pd(OAc)_2$[d]		0 (0)[e]

[a] All yields are based on purified product isolated by chromatography (SiO_2). [b] The % yields in parentheses are yields based on recovered, starting 1,1′-carbonyldipyrrole compound **14a** or **14b**. [c] 250 mg/mg of **14a**. [d] 500 mg/mg of **14b**. [e] **14c** = 1,1′-carbonyldipyrrole-2-carboxaldehyde.[17] Complete consumption of **14c** was observed and attributed to acid-catalyzed 2,2′-dipyrrylmethene formation.

(13) Kleinspehn, G. G.; Corwin, A. H. *J. Am. Chem. Soc.* **1954**, *76*, 5641. Battersby, A. R.; Hunt, E.; McDonald, E.; Paine, J. B., III; Saunders, J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1008.

(14) (a) 3-Methoxy-2-(methoxycarbonyl)pyrrole (**12a**) proved identical in all comparable respects with authentic material[14b] and distinctly different from the isomeric 4-methoxy-2-(methoxycarbonyl)pyrrole. (b) Rapoport, H.; Willson, C. D. *J. Am. Chem. Soc.* **1962**, *84*, 630.

(15) Trost, B. M.; Kinson, P. L. *J. Org. Chem.* **1972**, *37*, 1273. Boger, D. L.; Brotherton, C. E. *J. Org. Chem.* **1984**, *49*, 4050.

(16) Cairncross, A.; Roland, J. R.; Henderson, R. M.; Sheppard, W. A. *J. Am. Chem. Soc.* **1970**, *92*, 3187.

(17) Boger, D. L.; Patel, M. *J. Org. Chem.* **1987**, *52*, 2319.

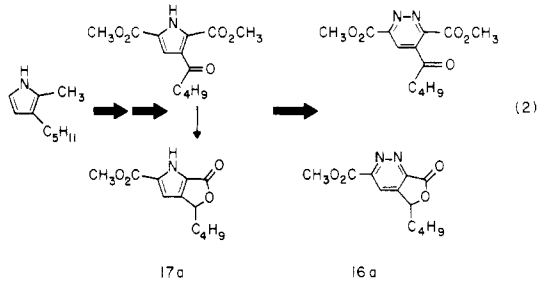
(18) (a) Tschelinzeff, W.; Maxoroff, B. *Chem. Ber.* **1927**, *60*, 144. Shirley, D. A.; Gross, B. H.; Roussel, P. A. *J. Org. Chem.* **1955**, *20*, 225. (b) Jung, M. E.; Rahloff, J. C. *J. Chem. Soc., Chem. Commun.* **1984**, 630.

(19) Appel, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 801. Barstow, L. E.; Hruby, V. J. *J. Org. Chem.* **1971**, *36*, 1305.

(20) (a) Itahara, T. *J. Chem. Soc., Chem. Commun.* **1980**, 49; **1981**, 254; *Heterocycles* **1980**, *14*, 100; *J. Org. Chem.* **1985**, *50*, 5272; *Synthesis* **1979**, 151. For related efforts, see: (b) Itahara, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 305; Akermark, B.; Eberson, L.; Jensson, E.; Petterssen, E. *J. Org. Chem.* **1975**, *40*, 1365. Bergman, J.; Eklund, N. *Tetrahedron* **1980**, *36*, 1439. Black, D. St. C.; Kumar, N.; Wong, L. C. H. *J. Chem. Soc., Chem. Commun.* **1985**, 1174. (c) Heck, R. F. *Palladium Reagents in Organic Synthesis*; Academic: Orlando, 1985; pp 180–184. (d) No reaction was observed when **14a** was treated with $(Ph_3P)_4Pd$ (0.1–1.0 equiv, benzene or acetic acid) confirming that palladium(II) is the oxidation state of the active palladium coupling reagent.

soluble palladium(II) acetate in acetic acid[20] or palladium(II) trifluoracetate[22] in trifluoroacetic acid[22] provided excellent conversion of **14a,b** to **15a,b** based on the stoichiometric use of reagent in limiting quantities. However, attempts to promote the complete consumption of substrates **14a,b** led to competitive reactions of product 2,2′-bipyrroles **15a,b** with reagent at the expense of increased 2,2′-bipyrrole coupling (Table I). Similar observations in efforts to effect intermolecular palladium(II)-promoted biaryl coupling reactions have been detailed.[20] The use of polymer-supported palladium(II) acetate[21] provided the 2,2′-bipyrrole coupling (**15a**, 96%; **15b**, 90%) with no evidence of competitive reaction of the electron-deficient 2,2′-bipyrroles with the polymer-supported reagent. Thus, the use of the polymer-supported palladium(II) acetate reagent provided an effective solution to the preparation of mixed, electron-deficient 2,2′-bipyrroles and potentially represents a general solution to the implementation of an effective palladium(II)-promoted 2,2′-diaryl coupling reaction. This contrasts the results of early, extensive efforts to improve the soluble palladium(II) acetate promoted 2,2′-bipyrrole coupling reaction employing catalytic, recycled reagent [0.01–0.1 equiv of $Pd(OAc)_2$; 1.0–3.0 equiv of $Cu(OAc)_2$, 0–10% **15b**][23,20d] and the unsuccessful efforts to employ alternative reagents [$Hg(OAc)_2$,[24] $Pb(OAc)_4$,[25] PbO_2,[26] NiO_2,[27] $Tl(OCOCF_3)_2$[28]] to promote the intramolecular 2,2′-bipyrrole coupling reaction of **14a,b**.

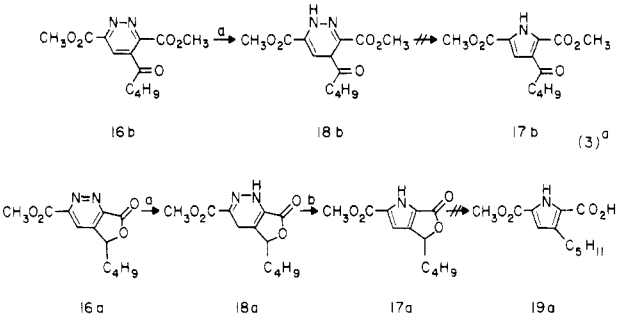
Efforts on the Preparation of Prodigiosin Ring C, 2-Methyl-3-pentylpyrrole: Further Studies on the Reductive Ring Contraction of Electron-Deficient 1,2-Diazines and Limitations of the 1,2,4,5-Tetrazine → 1,2-Diazine → Pyrrole Strategy. In the latter stages of efforts on the total synthesis of prodigiosin (**1**), it was anticipated that an extension of the 1,2,4,5-tetrazine → 1,2-diazine → pyrrole Diels–Alder strategy may permit the preparation of the prodigiosin ring C pyrrole, 2-methyl-3-pentylpyrrole, provided an effective differentiation of the C-2/C-5 methoxycarbonyl groups could be devised and successfully implemented (eq 2). Functionalization of the



(2)

C-4 1,2-diazine (pentyl) side chain provided an attractive solution to accomplishing the 1,2-diazine C-3/C-6 *and/or* pyrrole C-2/C-5 methoxycarbonyl differentiation.[29] The potential that 1,2-diazine C-3/C-6 methoxycarbonyl differentiation, C-4 pentyl introduction, and the 1,2-diazine to pyrrole reductive ring contraction could be achieved in one operation provided conditions could be devised that would permit the concurrent or subsequent[30] reductive cleavage of a benzylic lactone, cf. **16a/17a**, provided the basis for our investigations.

The [4 + 2] cycloaddition reaction of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**) with functionalized alkyne (Table II, entries 1–8) *or* alkene dienophiles (Table II, entry 9) provided the Diels–Alder cycloadducts in excellent yield. In the instances of the use of alkyne dienophiles bearing a free hydroxyl group, lactone formation was found to occur under the conditions required of the Diels–Alder reaction (five-membered lactone; Table II, entry 1), upon chromatographic purification (six-membered lactone; Table II, entry 3), or upon subsequent acid-catalyzed lactonization (seven-membered lactone; Table II, entry 5) of the [4 + 2] cycloadducts. Contrary to expectations, the zinc-promoted reductive ring contraction of the C-4 functionalized dimethyl 1,2-diazine-3,6-dicarboxylates failed to provide the corresponding pyrroles in acceptable yields. In each instance, the initial reduction of the electron-deficient 1,2-diazine proceeded rapidly and cleanly (10 equiv of Zn, HOAc, 25 C, 0.5–2 h) to provide a 1,4-dihydro-1,2-diazine (cf. **18a**),[31] and the rate of reaction could be accelerated by the use of trifluoroacetic acid. Under more vigorous reaction conditions (10–20 equiv of Zn, HOAc, 25 °C, 12–24 h), no evidence was secured to suggest that a benzylic lactone could be reductively cleaved under the reaction conditions required to promote the reductive ring contraction (eq 3). Initial attempts to



(3)[a]

[a](a) Zn, HOAc (CF_3CO_2H), 25°C, 0.5–2 h. (b) H_2/PtO_2, CH_3OH, 25°C.

promote the reductive ring contraction of the 1,2-diazine lactone **16a** [Zn, HOAc, 25 °C, 12–48 h; Al(Hg) or Na(Hg), 25 °C, moist THF] or the reduction and/or rearrangement of the isolated, characterized 1,4-dihydro-1,2-diazine lactone **18a** failed to provide the pyrrole lactone **17a**, pyrrolecarboxylic acid **19a**, or isomeric *N*-aminopyrrole. Although the reductive ring contraction of the 1,4-dihydro-

(21) The polymer-supported palladium(II) acetate was prepared as follows: Bio Rad Bio Beads SX-1 resin (polystyrene–1% divinylbenzene, 14 000 mol wt exclusion limit) was brominated and phosphinylated as detailed [Pittman, C. U., Jr.; Smith, L. R. *J. Am. Chem. Soc.* **1975**, *97*, 341], Anal. P, 1.45%, and subsequently loaded by treatment with palladium(II) acetate in benzene,[22] Anal. Pd, 2.79%.

(22) (a) Stephenson, T. A.; Morehouse, S. M.; Powell, A. R.; Heffer, J. P.; Wilkinson, G. *J. Chem. Soc.* **1965**, 3632. (b) Pittman, C. U., Jr.; Wuu, S. K.; Jacobson, S. E. *J. Catal.* **1976**, *44*, 87.

(23) Eglington, G.; McCrae, W. *Adv. Org. Chem.* **1963**, *4*, 225.

(24) Julia, M.; Colomer, E.; Julia, S. *Bull. Soc. Chim., Fr.* **1976**, 2397.

(25) Jones, S. R.; Mellor, J. M. *Synthesis* **1976**, 32.

(26) de Jonge, C. R. H. I.; Van Dort, H. M.; Vollbracht, L. *Tetrahedron Lett.* **1970**, 1881.

(27) George, M. V.; Balachandran, K. S. *Chem. Rev.* **1975**, *75*, 491.

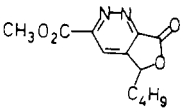
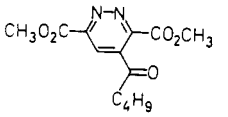
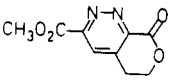
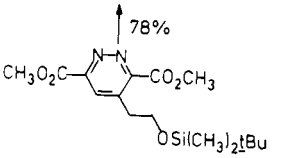
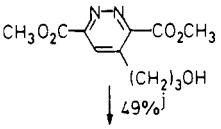
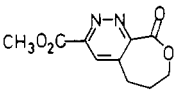
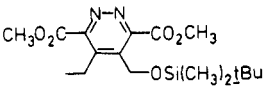
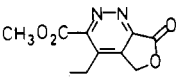
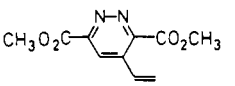
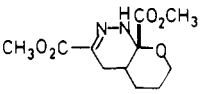
(28) Taylor, E. C.; Andrade, J. G.; Gerhardus, J. H. Rall; McKillop. A. *J. Am. Chem. Soc.* **1980**, *102*, 6513.

(29) Boger, D. L.; Panek, J. S.; Duff, S. R.; Yasuda, M. *J. Org. Chem.* **1985**, *50*, 5782, 5790. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1987**, *109*, 2717.

(30) Electron-deficient 1,2-diazines are reduced to the corresponding 1,4-dihydro-1,2-diazines under mild conditions [$NaBH_4$, EtOH, 25 °C;[10b] Zn, HOAc,[9,10] 25 °C]. Consequently, the reductive cleavage of a benzylic lactone was expected to occur subsequent to 1,2-diazine ring contraction.

(31) The 1,4-dihydro-1,2-diazines **18b** (10 equiv of zinc, CF_3CO_2H, 0–25 °C, 20 min) and **18c** (10 equiv of zinc, 0.5–2 h, HOAc, 25 °C) were cleanly and rapidly produced in the initial stages of the attempted reductive 1,2-diazine ring contraction reactions of **16b** and **16c**. For **18b**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.40 (br s, 1 H, NH), 5.90 (dd, 1 H, J = 1, 2 Hz, C=C*H*CH), 3.92 (d, 1 H, J = 2 Hz, C*H*COC_4H_9), 3.85 (s, 6 H, OCH_3), 2.54 (t, 2 H, J = 7 Hz, $COCH_2CH_2C_2H_5$), 1.57 (p, 2 H, J = 7 Hz, $COCH_2CH_2CH_2CH_3$), 1.37 (h, 2 H, J = 7 Hz, $COCH_2CH_2CH_2CH_3$), 0.89 (t, 3 H, J = 7 Hz, CH_3). For **18c**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 4.42 (t, 2 H, J = 6 Hz), 3.92 (s, 3 H), 3.87 (d, 2 H, J = 1 Hz), 2.93 (t, 2 H, J = 6 Hz), 2.52 (dt, 1 H, J = 2, 6 Hz).

Table II. Diels–Alder Reactions of Dimethyl 1,2,4,5-Tetrazine-3,6-dicarboxylate (7)

entry	dienophile	conditions:[a] equiv, temp (time)	1,2-diazine	yield, %
1	$CH_3(CH_2)_3CH(OH)C{\equiv}CH$	1.2, 80 °C (20 h)		**16a**, 84
2	$CH_3(CH_2)_3C(O)C{\equiv}CH$	1.2, 100 °C (12 h)		**16b**, 70
3[b]	$HOCH_2CH_2C{\equiv}CH$	1.2, 80 °C (6 h)		**16c**, 58
			78% ↑	
4	t-$Bu(Me)_2SiO(CH_2)_2C{\equiv}CH$	1.2, 80 °C (8 h)		**16d**, 79
5[c]	$HOCH_2(CH_2)_2C{\equiv}CH$	1.2, 50 °C (12 h)		**16e**, 83
			49% ↓	
				**16f**, 49
6[b]	t-$Bu(Me)_2SiOCH_2C{\equiv}CEt$	1.2, 140 °C (2.5 h)[d]		**16g**, 54
			79% ↓	
7	$HOCH_2C{\equiv}CEt$	1.2, 50 °C (18 h)		**16h**, 46
8[e]	$PhCH_2O(CH_2)_2C{\equiv}CH$	1.2, 101 °C (12 h)		**16i**, 60
9	$HO(CH_2)_3CH{=}CH_2$	1.2, 25 °C (1 h)		**16j**, 92

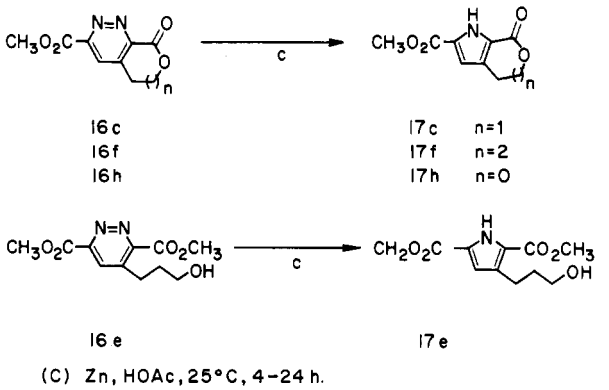
[a] All [4 + 2] cycloaddition reactions were conducted in dioxane (0.3–0.5 M) under an atmosphere of nitrogen. [b] Lactonization occurs upon deprotection of **16d** [HOAc–THF–H_2O (3:1:1), 25 °C, 12 h]. [c] Closure to the lactone was effected by *p*-toluenesulfonic acid (catalyst), benzene, 80 °C, 12–15 h, with azeotropic removal of methanol. [d] The Diels–Alder reaction was conducted in mesitylene (0.5 M) under an atmosphere of nitrogen. [e] Loss (elimination) of benzyl alcohol was observed subsequent to the initial [4 + 2] cycloaddition reaction under the reaction conditions and upon attempted purification of the [4 + 2] cycloadduct.

Table III. In Vitro Cytotoxic Activity

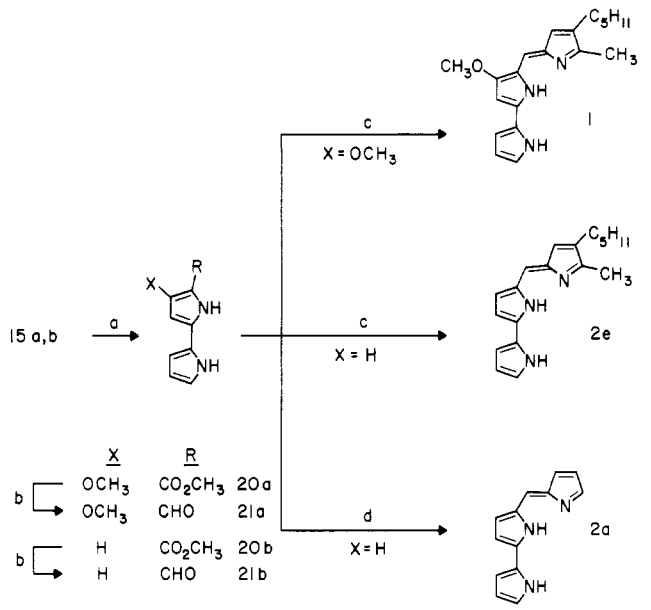
	IC_{50}, μg/mL[a]			
	L1210[b]	B16[c]	9PS(P388)[d]	9KB[e]
1	0.02	0.03	0.000 37	0.04
2a	17	6	0.07	6.4
2e	12	24	0.03	0.7

[a] Inhibitory concentration for 50% cell growth relative to untreated control (IC_{50}). [b] L-1210 mouse lymphocytic leukemia cell culture. [c] B16 mouse melanoma cell culture. [d] P388 mouse leukemia cell culture. [e] Human epidermoid carcinoma of the nasopharynx.

1,2-diazine lactone **18a** could be effected by employing catalytic hydrogenation (H_2/PtO_2 catalyst, THF or CH_3OH, 25 °C, 6–20 h, 10–21% unoptimized; H_2/10% Pd–C catalyst, CH_3OH, 25 °C, 12–24 h) and provided the pyrrole lactone **17a**, no conditions were found to effect the concurrent benzylic lactone cleavage. Less direct approaches to methoxycarbonyl differentiation, 1,2-diazine C-4 (pyrrole C-3) pentyl introduction, and 1,2-diazine to pyrrole reductive ring contraction employing the 1,2-diazine lactones **16c,f** were not encouraging and discouraged continued investigation of this approach to 2-methyl-3-pentylpyrrole.



Scheme IV[a]



[a] (a) $LiOCH_3$, CH_3OH; 88% **20a**, 95% **20b**; (b) NH_2NH_2, 98%, 100%; *p*-TsCl, pyridine, 97%, 98%; Na_2CO_3, $HOCH_2CH_2OH$, 170 °C, 34% overall for **21a**; 39% overall for **21b**; (c) 1.0 equiv of 2-methyl-3-pentylpyrrole, CH_3OH, catalytic HCl, 59% for **1**; catalytic HBr, 44% for **2e**; (d) pyrrole, CH_3OH, catalytic HBr, 36%.

Total Synthesis of Prodigiosin (1), Prodigiosene (2a), and 2-Methyl-3-Pentylprodigiosene (2e, Desmethoxyprodigiosin). Mild methanolysis of the labile urea of the 1,1′-carbonyl-2,2′-bipyrrole derivatives **15a,b** provided **20a,b** and completed the preparation of the prodigiosin and prodigiosene 2,2′-bipyrrole AB ring systems, respectively (Scheme IV). The final conversion of **20a** to prodigiosin (**1**) via aldehyde **21a** and its acid-catalyzed condensation with 2-methyl-3-pentylpyrrole[32] followed the conditions developed in the total syntheses of prodigiosin detailed by Rapoport and Wasserman.[1] Aldehyde **21a** possessed the spectroscopic and physical properties described for synthetic,[1c,d] naturally derived,[1b] and naturally occurring material.[1b] Synthetic prodigiosin (**1**) and its hydrochloride proved identical in all comparable respects with samples of authentic, natural prodigiosin [^1H NMR (200 MHz), IR, EI/CIMS, HRMS, TLC (50% CH_2Cl_2–Et_2O; 50% EtOAc–hexane; 75% Et_2O–hexane)] and prodigiosin hydrochloride [^1H NMR (200 MHz)].

Condensation of 2,2′-bipyrrole-5-carboxaldehyde (**21b**) with pyrrole and 2-methyl-3-pentylpyrrole[32] provided prodigiosene (**2a**) and 2-methyl-3-pentylprodigiosene (**2e**, desmethoxyprodigiosin), respectively. The pyrrolylpyrromethene condensation of **21b** with pyrrole and 2-methyl-3-pentylpyrrole proved competitive with that observed in the preparation of prodigiosin although the pyrrolylpyrromethene condensation products (**2a,e**) proved less stable to chromatographic purification and storage. The properties of synthetic prodigiosene (**2a**) were identical with those reported for naturally occurring[2] and synthetic material.[4]

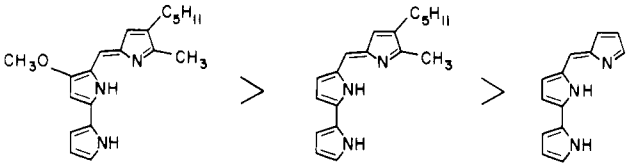


Figure 1.

In Vitro Antimicrobial and Cytotoxic Activity. Prodigiosin (**1**), prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosene (**2e**, desmethoxyprodigiosin) were subjected to comparative in vitro cytotoxic and antimicrobial[33] evaluation in efforts to define the role the prodigiosin peripheral substituents play in contributing to or potentiating the observed properties of the prodigiosenes. The in vitro cell culture cytotoxic assays were performed by employing B16 mouse melanoma,[34] L1210 mouse leukemia,[34] P388 mouse leukemia (9PS),[35] and human epidermoid carcinoma of the nasopharynx (9KB)[35] following established protocols. The results [inhibitory concentration for 50% cell growth relative to untreated controls (IC_{50}, μg/mL)] are detailed in Table III. Antimicrobial assays were performed by using an agar dilution/streak assay against seven microorganisms: *Staphylococcus aureus* ATCC 13709, *Escherichia coli* ATCC 9637, *Salmonella gallinarum* ATCC 9184, *Klebsiella pneumoniae* ATCC 10031, *Mycobacterium smegmatis* ATCC 607, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231; representing gram-positive, gram-negative, acid-fast, and fungal microorganisms.[33b]

The sequential removal of the prodigiosin peripheral substituents diminished the observed cytotoxic potency of the resulting agents (**1** ≫ **2e** ≥ **2a**; Figure 1). Prodigiosin (**1**) was found to be exceptionally potent in vitro against P388 leukemia ($ID_{50} = 3.7 \times 10^{-4}$ μg/mL = 3.7×10^{-10} g/mL) and displayed substantial in vitro cytotoxic activity against L1210, B16, and 9KB cell lines (ID_{50} = 0.02, 0.03, and 0.04 μg/mL, respectively), which may be attributed to the presence of the peripheral prodigiosene C-6 methoxy substituent.

In efforts to determine whether the C-6 methoxy potentiation of the biological properties may be related to an influence in the conformation or chemical reactivity of the prodigiosenes, a structural and electronic comparison of prodigiosin (**1**) and 2-methyl-3-pentylprodigiosin (**2e**) was undertaken.[36] Employing MM2 (CHEMLAB-II,

(32) (a) 2-Methyl-3-pentylpyrrole[1e] was prepared in a modification of a previously detailed procedure from 5-(ethoxycarbonyl)-2-methyl-3-valeroylpyrrole[1e] by the following sequence: (i) 5.0 equiv of LiOH, THF–H_2O–CH_3OH (3:1:1), 45 °C, 12 h, 79–84%; (ii) 5.6 equiv of NaI, 6.2 equiv of I_2, 9.1 equiv of $NaHCO_3$, H_2O–$ClCH_2CH_2Cl$ (1:1), 25 °C, 6 h, 89%; 10% Pd/C, 2.0 equiv of K_2CO_3, CH_3OH, H_2 (1 atm), 25 °C, 3 h, 93% (–CO_2); (iii) NH_2NH_2 (2 equiv of 85%), 3.5 equiv of KOH, $HOCH_2CH_2OH$, 175 °C, 2.5 h, 62%.

(33) (a) Minimum inhibitory concentration (MIC, μg/mL) were determined by using the agar-dilution/streak method [cf.: Mitscher, L. A.; Leu, R.-P.; Bathala, M. S.; Wu, W.-N.; Beal, J. L.; White, R. *Lloydia* **1972**, *35*, 157] by Steven D. Drake, Department of Medicinal Chemistry, University of Kansas. (b) Prodigiosin (**1**), prodigiosene (**2a**), and 2-methyl-3-pentylprodigiosin (**2e**) were inactive (MIC > 20 μg/mL) against all seven microorganisms tested.

(34) Cell culture IC_{50} (μg/mL, inhibitory concentration for 50% cell growth relative to untreated control) determinations for B16 mouse melanoma and L1210 mouse leukemia were determined by Professor P. A. Kitos and S. Collins, Department of Biochemistry, University of Kansas, employing a previously detailed procedure, cf.: Boger, D. L.; Mitscher, L. A.; Mullican, M. D.; Drake, S. D.; Kitos, P. A. *J. Med. Chem.* **1985**, *28*, 1543.

(35) Cell culture IC_{50} (μg/mL) determinations for P388 (9PS) mouse leukemia and human epidermoid carcinoma of the nasopharynx (9KB) were determined under the supervision of the Purdue University Cancer Center Cell Culture Laboratory following the protocols established by the National Institutes of Health, National Cancer Institute, cf.: Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3* (2), 17–20, 59–61.

version 9.0) optimized geometries as the starting geometries,[36b] INDO (CHEMLAB-II, version 9.0) optimization of prodigiosin (1) and 2-methyl-3-pentylprodigiosene (**2e**) free base revealed no substantial structural or electronic differences that might be readily correlated to the relative cytotoxic potency of the two agents (Table IV, supplementary material). The magnitude of the LUMO coefficients for both agents was found to be consistent with preferential nucleophilic attack at the C-5 methene carbon, an established chemical behavior of the prodigiosenes.[4a,5a] In a refined comparison employing further optimization of the two agents (AMPAC, AM1 Hamiltonian, version 1.0 and 3.11) and final, single geometry SCF calculation with C.I. (AMPAC, AM1 Hamiltonian, C.I. = 5 for 1/C.I. = 6 for **2e**), the observed differences (ΔE HOMO, ΔE LUMO, $\Delta\Delta E$ HOMO/LUMO) in the two agents were found to be more pronounced but not of a sufficient magnitude to readily correlate to the selected, potent cytotoxic activity of prodigiosin (1).[36d] Consequently, the role of the prodigiosin C-6 methoxy substituent does not appear to be related to a direct, conformational or electronic effect on the prodigiosene structure or chemical reactivity.

Experimental Section

General Experimental Details. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian FT-80A or a Varian XL-200 and chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane (0.00 ppm). Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on a Varian XL-200 and are reported in ppm relative to tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on an IBM FTIR 32, Perkin-Elmer FTIR 1710, Perkin-Elmer 1420, or a Perkin-Elmer FTIR 1800 as KBr pellets (solids) or thin films (liquids). Melting points (mp) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Electron impact mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were recorded on a Varian CH-5 or a Finnegan 4000 mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a Ribermag R10-10 or a Kratos MS-50 mass spectrometer. Tetrahydrofuran (THF) and ether (Et_2O) were distilled from sodium benzophenone ketyl. *N,N*-Dimethylformamide (DMF) and dioxane were distilled from calcium hydride. Methylene chloride (CH_2Cl_2) was distilled from phosphorus pentoxide. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI·HCl) was obtained from Aldrich Chemical Co. All reactions were performed under a positive atmosphere of nitrogen (N_2) or argon. Neutral alumina (activity grade III) was prepared from neutral alumina (activity grade I) by the addition of water (6% w/w). Column chromatography was performed on silica gel 60 (70–230 mesh, ASTM).

Dimethyl 4-Methoxy-1,2-diazine-3,6-dicarboxylate (8). 1,1-Dimethoxyethylene (0.49 mL, 5.1 mmol)[12] was added dropwise to a solution of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 840 mg, 4.2 mmol)[11] in dioxane (17 mL) at 25 °C, and the reaction mixture was stirred at 25 °C for an additional 3 h before the solvent was removed in vacuo. Chromatography (SiO_2, 4 cm × 15 cm, ether eluant) afforded **8** (901 mg, 958 mg theoretical, 94%) as a pale yellow solid: mp 104–105 °C (methanol, pale yellow needles); ^{1}H NMR ($CDCl_3$, 80 MHz, ppm) 7.74 (s, 1 H), 4.08 (s, 3 H), 4.05 (s, 3 H), 4.04 (s, 3 H); IR (KBr) ν_{max} 2955, 1748, 1728, 1568, 1447, 1306, 1242, 1136, 1021 cm^{-1}; EIMS, *m/e* (relative intensity) 226 (M^+, 6), 195 (16), 168 (54), 138 (13), 109 (base); HRMS, *m/e* 226.0600 ($C_9H_{10}N_2O_5$ requires 226.0589).

Dimethyl 3-Methoxypyrrole-2,5-dicarboxylate (9). Zinc dust (5.75 g, 88.5 mmol, 10 equiv) was added to a solution of **8** (2.0 g, 8.85 mmol) in acetic acid (105 mL) at 25 °C, and the resulting reaction mixture was stirred at 25 °C for 5 h. The reaction mixture was filtered through Celite, and the filtrate was made basic with the addition of 10% aqueous ammonium hydroxide. The aqueous phase was extracted with ethyl acetate (6 × 100 mL), and the combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 4 cm × 25 cm, ether eluant) afforded **6** (1.28 g, 1.88 g theoretical, 68%) as a white solid: mp 149.5–150.5 °C (methanol, white needles); ^{1}H NMR ($CDCl_3$, 80 MHz, ppm) 9.30 (br, s, 1 H), 6.51 (d, 1 H, J = 3 Hz), 3.89 (s, 6 H), 3.88 (s, 3 H); IR (KBr) ν_{max} 3289, 3006, 2957, 1721, 1680, 1570, 1514, 1437, 1283, 1229 cm^{-1}; EIMS, *m/e* (relative intensity) 213 (M^+, 98), 198 (6), 180 (26), 166 (13), 153 (77), 150 (99), 138 (19), 123 (72), 94 (37), 65 (32), 59 (30), 53 (base); HRMS, *m/e* 213.0634 ($C_9H_{11}NO_5$ requires 213.0636).

3-Methoxy-2-(methoxycarbonyl)pyrrole-5-carboxylic Acid (10). Lithium hydroxide (331 mg, 7.89 mmol, 1.0 equiv) was added to a solution of **9** (1.68 g, 7.89 mmol) in THF/CH_3OH/H_2O (3:1:1, 30 mL) at 25 °C, and the resulting reaction mixture was allowed to stir at 25 °C for 78 h. The reaction mixture was poured onto water (20 mL) and extracted with ether (2 × 20 mL). The aqueous phase was acidified to pH 1 with the addition of 10% aqueous hydrochloric acid and extracted with ethyl acetate (6 × 100 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated in vacuo to afford pure **10** (1.43 g, 1.57 g, theoretical, 91%): mp 182–184 °C dec (methanol, white platelets); ^{1}H NMR (CD_3COCD_3, 80 MHz, ppm) 6.72 (br s, 1 H), 6.57 (d, 1 H, J = 4 Hz), 3.84 (s, 3 H), 3.78 (s, 3 H); IR (KBr) ν_{max} 3293, 3280, 3276, 3260, 3194, 3023, 2990, 2921, 2888, 1720, 1707, 1667, 1569, 1517, 1426, 1279, 1132 cm^{-1}; EIMS, *m/e* (relative intensity) 200 (M^+ + 1, 10), 199 (M^+, base), 181 (26), 168 (15), 167 (43), 166 (33), 153 (56), 150 (82), 148 (16), 124 (45), 123 (34), 122 (10), 94 (16); HRMS, *m/e* 199.0451 ($C_8H_9NO_5$ requires 199.0481).

Anal. Calcd for $C_8H_9NO_5$: C, 48.24; H, 4.56; N, 7.03. Found: C, 48.14; H, 4.72; N, 6.77.

4,5-Diiodo-3-methoxy-2-(methoxycarbonyl)pyrrole (11). A solution of NaI (132 mg, 0.89 mmol) and I_2 (247 mg, 0.98 mmol) in water (0.58 mL) was added to a two-phase solution of **10** (32 mg, 0.16 mmol) and $NaHCO_3$ (124 mg, 1.47 mmol) in $ClCH_2CH_2Cl$/H_2O (1:1, 0.6 mL), and the resulting mixture was allowed to stir at 25 °C for 12 h protected from light. The reaction mixture was treated with solid sodium bisulfite until the red color of iodine disappeared and subsequently was extracted with ether (2 × 25 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 2 cm × 10 cm, ether eluant) afforded **11** (58 mg, 65 mg theoretical, 89%) as a white solid: mp 162–163 °C (hexane, white needles); ^{1}H NMR (CD_3COCD_3, 80 MHz, ppm) 3.84 (s, 3 H), 3.79 (s, 3 H); IR (KBr) ν_{max} 3237, 3166, 2934, 1681, 1489, 1438, 1387, 1248, 1195, 1184, 1130, 1116, 996, 963, 948, 910, 895, 878 cm^{-1}; EIMS, *m/e* (relative intensity) 407 (M^+, 82), 375 (base), 360 (15), 332 (25), 225 (13), 220 (37), 179 (75), 127 (28).

3-Methoxy-2-(methoxycarbonyl)pyrrole (12a). A solution of **11** (500 mg, 1.22 mmol), potassium carbonate (336 mg, 2.44 mmol), and 5% Pd/C (50 mg) in methanol (5 mL) was allowed to stir under an atmosphere of hydrogen at 25 °C for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. Chromatography (SiO_2, 3 × 15 cm, ether eluant) afforded **12a** (182 mg, 189 mg theoretical, 96%) as a white solid: mp 112–114 °C (EtOAc–hexane, white needles); ^{1}H NMR ($CDCl_3$, 80 MHz, ppm) 9.22 (br s, 1 H), 6.80 (t, 1 H, J = 2 Hz), 5.90 (t, 1 H, J = 2 Hz), 3.90 (s, 3 H), 3.87 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 161.5 (s), 152.9 (s), 121.6 (d), 106.7 (s), 95.9 (d),

(36) (a) Consistent with experimental and computational evidence[36b] including but not limited to a strong hydrogen bond between the prodigiosin B and C pyrrole rings, structure 1 bearing the *Z* olefin geometry best approximates the structure of prodigiosin free base. Spectral properties of selected, protonated prodigiosenes (cf.: **2e**, 2-methyl-3-pentylprodigiosene) suggests they may exist as mixtures of distinct *E,Z* olefinic isomers.[5a] (b) Boger, D. L.; Severance, D., unpublished observations. MM2 optimized geometries, (Allinger, MM2, MacroModel, version 1.1 and CHEMLAB-II, version 9.0) of *E/Z*-1, *E/Z*-**2a**, and *E/Z*-**2e** in linear as well as the represented nonlinear conformation were examined. In each instance, the nonlinear *Z* conformation represents the lower energy conformation. (c) No correlation of pK_a (**1**, 8.25; **20**, 7.20; **2e**, 8.35) and cytotoxic activity is apparent. (d) Similarly, INDO optimization of protonated prodigiosin and protonated **2e** revealed no substantial structural or electronic differences that might be readily correlated to the relative cytotoxic potency of the two agents.

(37) Attempts to improve the McFayden–Stevens reduction employing recent experimental variants proved unsuccessful, see: Dudman, C. C.; Grice, P.; Reese, C. B. *Tetrahedron Lett.* **1980**, 4645.

58.3 (q), 51.3 (q); IR (KBr) ν_{max} 3385, 3364, 3296, 3134, 2941, 2461, 1718, 1666, 1564, 1514, 1446, 1412, 1327, 1288, 1230, 1186, 1142, 1107, 1080, 1012 cm^{-1}; UV (CH_3OH) λ_{max} 265 nm (ϵ 18630) [lit.[14b] UV (CH_3OH) λ_{max} 265 nm (ϵ, not reported)]; EIMS, *m/e* (relative intensity) 155 (M^+, 66), 124 (42), 123 (base), 122 (15), 108 (14), 80 (43); HRMS, *m/e* 155.0563 ($C_7H_9NO_3$ requires 155.0582).

3-Methoxy-2-(methoxycarbonyl)-1,1'-carbonyldipyrrole (14a). Method A. The sodium salt of 3-methoxy-2-(methoxycarbonyl)pyrrole [generated in THF (0.5 mL) at 65 °C for 14 h from 3-methoxy-2-(methoxycarbonyl)pyrrole (26 mg, 0.167 mmol) and NaH (8 mg of 50% dispersion in mineral oil, 0.167 mmol)] was added to a solution of pyrrole-1-carboxylic anhydride[17] (41 mg, 0.2 mmol) in tetrahydrofuran (0.5 mL) at 25 °C, and the resulting mixture was allowed to stir for 15 min. The reaction was poured onto 5% aqueous sodium bicarbonate and extracted with ether (2 × 10 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 1 cm × 15 cm, 15% ether–hexane eluant) afforded **14a** (22 mg, 42 mg theoretical, 52%).

Method B. Triphenylphosphine (50 mg, 0.19 mmol) was added to a solution of pyrrole-1-carboxylic acid (17 mg, 0.16 mmol) and carbon tetrachloride (0.076 mL, 0.8 mmol)[17] in acetonitrile (0.3 mL), and the reaction mixture was allowed to stir at 25 °C for 2.5 h. The sodium salt of 3-methoxy-2-methoxycarbonyl)pyrrole [generated in THF (0.5 mL) at 65 °C (14 h) from 3-methoxy-2-(methoxycarbonyl)pyrrole (50 mg, 0.32 mmol), NaH (15 mg of 50% dispersion in mineral oil, 0.32 mmol) and 18-crown-6 (84.5 mg, 0.32 mmol)], was added. The reaction mixture was stirred at 25 °C for 15 min, poured onto water (15 mL), and extracted with ether (2 × 25 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 1 cm × 20 cm, 15% ether–hexane eluant) afforded **14a** (28 mg, 44 mg theoretical, 64%) as a yellow oil: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.16 (t, 2 H, J = 2 Hz), 7.11 (d, 1 H, J = 3 Hz), 6.32 (t, 2 H, J = 2 Hz), 6.15 (d, 1 H, J = 3 Hz), 3.94 (s, 3 H), 3.69 (s, 3 H); IR (KBr) ν_{max} 3140, 2940, 1740, 1700, 1570, 1450, 1400, 1340, 1260, 1090, 840 cm^{-1}; EIMS, *m/e* (relative intensity) 248 (M^+, 23), 217 (1), 182 (base), 154 (1), 123 (10), 94 (14), 66 (21); CIMS (2-methylpropane), *m/e* 249 (M^+ + H, base); HRMS, *m/e* 248.0801 ($C_{12}H_{12}N_2O_4$ requires 248.0797).

2-(Methoxycarbonyl)-1,1'-carbonyldipyrrole (14b). Method A. Oxalyl chloride (0.78 mL, 6.2 mmol) was added to a solution of pyrrole-1-carboxylic acid[17] (348 mg, 3.1 mmol) in tetrahydrofuran (2 mL) at 25 °C containing catalytic *N,N*-dimethylformamide (15 μL), and the reaction mixture was allowed to stir at 25 °C until evolution of gases ceased (ca. 10 min). The reaction mixture was concentrated in vacuo, and the crude pyrrole-1-carboxylic acid chloride was taken up to tetrahydrofuran (2 mL). The sodium salt of methyl pyrrole-2-carboxylate [generated in THF (2 mL) at 25 °C from methyl pyrrole-2-carboxylate (200 mg, 1.6 mmol) and NaH (77 mg of 50% dispersion in mineral oil, 1.6 mmol)] was added. The reaction mixture was stirred at 25 °C for 15 min, poured onto water, and extracted with ether (3 × 50 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 3 cm × 20 cm, 25% ether–hexane eluant) afforded **14b** (310 mg, 349 mg theoretical, 89%) as a pale yellow solid: mp 61–61.5 °C (hexane, platelets); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.24 (m, 1 H), 7.10 (m, 3 H), 6.36 (m, 3 H), 3.72 (s, 3 H); IR (KBr) ν_{max} 3140, 2940, 1730, 1710, 1540, 1470, 1440, 1400, 1350, 1280, 1240, 1120, 1090, 1070, 1030, 980, 940, 890, 860, 790, 760, 740 cm^{-1}; EIMS, *m/e* (relative intensity) 218 (M^+, 16), 187 (5), 159 (2), 153 (5), 152 (base), 137 (1), 125 (3), 111 (4), 94 (23), 80 (19), 66 (38); CIMS (2-methylbutane), *m/e* 219 (M^+ + H, base); HRMS, *m/e* 218.0685 ($C_{11}H_{10}N_2O_3$ requires 218.0691).

Anal. Calcd for $C_{11}H_{10}N_2O_3$: C, 60.54; H, 4.62; N, 12.84. Found: C, 60.62; H, 4.63; N, 12.80.

Method B. The sodium salt of methyl pyrrole-2-carboxylate [generated in THF (0.5 mL) at 25 °C from methyl pyrrole-2-carboxylate (50 mg, 0.4 mmol) and NaH (19 mg of 50% dispersion in mineral oil, 0.4 mmol)] was added to a solution of pyrrole-1-carboxylic anhydride[17] (98 mg, 0.48 mmol) in tetrahydrofuran (0.5 mL) at 25 °C, and the resulting mixture was allowed to stir at 25 °C for 15 min. The reaction mixture was poured onto 5% aqueous sodium bicarbonate and extracted with ether (2 × 10 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 2 cm × 10 cm, 25% ether–hexane eluant) afforded **14b** (61 mg, 88 mg theoretical, 69%).

4-Methoxy-5-(methoxycarbonyl)-1,1'-carbonyl-2,2'-bipyrrole (15a). Polymer bound palladium acetate (1.5 g, 2.79% Pd)[21] was added to a 25 °C solution of the carbonyl dipyrrole **14a** (6.1 mg, 0.024 mmol) in acetic acid (3 mL) at 25 °C, and the resulting slurry was warmed at 80 °C for 12 h. The reaction mixture was cooled to room temperature, filtered through Celite, and concentrated in vacuo. Chromatography (SiO_2, 1 cm × 2 cm, ether eluant) afforded **15a** (5.8 mg, 6.0 mg theoretical, 96%) as a yellow solid: mp 201–201.5 °C dec (hexane, bright yellow needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.05 (m, 1 H), 6.20 (m, 2 H), 6.05 (s, 1 H), 3.95 (s, 3 H), 3.85 (s, 3 H); IR (KBr) ν_{max} 3333, 3126, 3109, 2926, 1773, 1686, 1635, 1563, 1464, 1376, 1269, 1182, 1118, 1088, 1016, 989, 828, 776, 683 cm^{-1}; EIMS, *m/e* (relative intensity) 247 (M^+ + 1, 11), 246 (M^+, base), 215 (21), 201 (10), 188 (27), 173 (4), 159 (3), 145 (7), 129 (2), 116 (5), 102 (2), 90 (11); CIMS (2-methylpropane), *m/e* 247 (M^+ + H, base); HRMS, *m/e* 246.0635 ($C_{12}H_{10}N_2O_4$ requires 246.0640).

5-(Methoxycarbonyl)-1,1'-carbonyl-2,2'-bipyrrole (15b). Polymer bound palladium acetate (2.5 g, 2.79% Pd)[21] was added to a solution of the carbonyl dipyrrole **14b** (5.0 mg, 0.02 mmol) in acetic acid (3 mL) at 25 °C, and the resulting slurry was warmed at 80 °C for 36 h. The reaction mixture was cooled to room temperature, filtered through Celite, and concentrated in vacuo. Chromatography (SiO_2, 1 cm × 2 cm, ether eluant) afforded **15b** (4.5 mg, 4.95 mg theoretical, 90%) as a yellow solid: mp 104–105 °C (hexane, bright yellow needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.01 (d, 1 H, J = 3 Hz), 6.93 (d, 1 H, J = 3.8 Hz), 6.17 (m, 2 H), 6.06 (d, 1 H, J = 3.8 Hz), 3.87 (s, 3 H); IR (KBr) ν_{max} 3090, 1780, 1720, 1470, 1450, 1350, 1270, 1230, 1190, 1100, 1080, 810, 740 cm^{-1}; EIMS, *m/e* (relative intensity) 216 (M^+, base), 185 (41), 158 (19), 157 (19), 130 (14), 129 (19), 103 (15), 102 (19); CIMS (2-methylpropane), *m/e* 217 (M^+ + H, base); HRMS, *m/e* 216.0532 ($C_{11}H_8N_2O_3$ requires 216.0535).

5,7-Dihydro-3-(methoxycarbonyl)-5-*n*-butyl-7-oxofuro[3,4-*c*]pyradazine (16a). 1-Heptyn-3-ol (0.38 mL, 3.03 mmol)[12] was added to a solution of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 500 mg, 2.53 mmol)[11] in dioxane (10 mL) at 25 °C, and the reaction mixture was stirred at 80 °C for 20 h. The solvent was removed in vacuo. Chromatography (SiO_2, 4 cm × 15 cm, ether eluant) afforded **16a** (531 mg, 633 mg theoretical, 84%) as a yellow solid: mp 131–132 °C (hexane–EtOAc, 1:1, white platelets); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.38 (s, 1 H, 5.64 (dd, 1 H, J = 5.3 Hz), 4.15 (s, 3 H), 2.00 (m, 2 H), 1.52 (m, 2 H), 1.34 (m, 2 H), 0.93 (t, 3 H, J = 7 Hz); IR (KBr) ν_{max} 3065, 2955, 2860, 1784, 1740, 1591, 1460, 1441, 1327, 1286, 1115, 1057, 960 cm^{-1}; EIMS, *m/e* (relative intensity) 250 (M^+, 11), 220 (13), 192 (base), 148 (29), 120 (41), 105 (82), 92 (23), 77 (80); HRMS, *m/e* 250.0949 ($C_{12}H_{14}N_2O_4$ requires 250.0954).

Anal. Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.59; H, 5.81; N, 11.52.

Dimethyl 4-(1-Oxopentyl)-1,2-diazine-3,6-dicarboxylate (16b). Following the procedure for the preparation of **16a** [1-heptyn-3-one (0.52 mL, 4.24 mmol)[12] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 700 mg, 3.54 mmol);[11] Table II] provided 696 mg (991 mg theoretical, 70%) of **16b**: mp 68.5–69.5 °C (hexane–EtOAc, 1:1, yellow needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.21 (s, 1 H), 4.12 (s, 3 H), 4.08 (s, 3 H), 2.91 (t, 2 H, J = 7 Hz), 2.64 (m, 2 H), 2.30 (m, 2 H), 0.96 (t, 3 H, J = 7 Hz); IR (KBr) ν_{max} 2959 , 1734, 1442, 1379, 1294, 1259, 1197, 1142, 962, 916, 733, 646 cm^{-1}; EIMS, *m/e* relative intensity) 280 (M^+, 1), 249 (10), 238 (10), 221 (40), 210 (14e, 193 (32), 138 (57), 91 (11), 79 (59), 57 (base); HRMS, *m/e* 280.1057 ($C_{13}H_{16}N_2O_5$ requires 280.1059).

5,8-Dihydro-3-(methoxycarbonyl)-8-oxo-6*H*-pyrano[3,4-*c*]pyridazine (16c). Following the procedure for the preparation of **16a** [3-butyn-1-ol (53 μL, 0.54 mmol)[12] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 90 mg, 0.45 mmol);[11] Table II] provided 55 mg (94 mg theoretical, 58%) of **16c**: mp 186–188 °C dec (EtOAc–EtOH, 1:1, yellow platelets); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.18 (s, 1 H), 4.66 (t, 2 H, J = 5 Hz), 4.13 (s, 3 H), 3.29 (t, 2 H, J = 5 Hz); IR (KBr) ν_{max} 3066, 1734, 1583, 1487, 1412, 1333, 1287, 1149, 1067, 981, 894, 735, 666 cm^{-1}; EIMS, *m/e* (relative intensity) 208 (M^+, 5), 178 (7), 150 (base), 106 (26), 79 (36); CIMS

(2-methylpropane), *m/e* 209 (M^+ + H, base); HRMS, *m/e* 208.0457 ($C_9H_8N_2O_4$ requires 208.0484).

Anal. Calcd for $C_9H_8N_2O_4$: C, 51.92; H, 3.87; N, 13.46. Found: C, 52.09; H, 3.95; N, 13.31.

Dimethyl 4-[2-((*tert*-Butyldimethylsilyl)oxy)ethyl]-1,2-diazine-3,6-dicarboxylate (16d). Following the procedure for the preparation of **16a** [1-[(*tert*-butyldimethylsilyl)oxy]-3-butyne (0.31 mL, 1.52 mmol)[12] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 250 mg, 1.26 mmol); Table II] provided 350 mg (446 mg theoretical, 79%) of **16d**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.24 (s, 1 H), 4.09 (s, 3 H), 4.07 (s, 3 H), 3.93 (t, 2 H, *J* = 5 Hz), 3.15 (t, 2 H, *J* = 5 Hz), 0.82 (s, 9 H), 0.05 (s, 6 H); IR (KBr) ν_{max} 3066, 2958 1735, 1587, 1475, 1327, 1288, 1197, 1030, 948, 800, 735, 666 cm^{-1}; EIMS, *m/e* (relative intensity) 297 (M^+ – *t*-Bu, 77), 253 (3), 149 (2), 89 (base), 73 (15), 59 (20); CIMS (2-methylpropane), *m/e* 355 (M^+ + H, base); HRMS, *m/e* 354.1611 ($C_{16}H_{26}N_2O_5Si$ requires 354.1612).

Dimethyl 4-(3-Hydroxy-1-propyl)-1,2-diazine-3,6-dicarboxylate (16e). Following the procedure for the preparation of **16a** [4-pentyn-1-ol (0.65 mL, 6.9 mmol);[11] Table II] provided 1.21 g (1.46 g, theoretical, 83%) of **16e**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.18 (s, 1 H), 4.08 (s, 3 H), 4.06 (s, 3 H), 3.70 (apparent br t, 2 H, *J* = 3 Hz), 3.06 (t, 2 H, *J* = 7 Hz), 1.95 (p, 2 H, *J* = 7 Hz); IR (KBr) ν_{max} 3400, 3066, 2958, 2892, 1735, 1583, 1440, 1394, 1271, 1191, 1095, 989, 912, 826, 729, 714 cm^{-1}; EIMS, *m/e* (relative intensity) 254 (M^+, 3), 223 (10), 196 (20), 152 (base), 137 (14), 119 (26), 92 (24), 59 (70); CIMS (2-methylpropane), *m/e* 255 (M^+ + H, base); HRMS, *m/e* 254.0899 ($C_{11}H_{14}N_2O_5$ requires 254.0903).

5,6,7,9-Tetrahydro-3-(methoxycarbonyl)-9-oxooxepino[3,4-*c*]pyridazine (16f). *p*-Toluenesulfonic acid (21 mg, 0.11 mmol) was added to a slurry of lactone **16e** (282 mg, 1.11 mmol) in benzene (50 mL), and the resulting solution was warmed at reflux with azeotropic removal of methanol (12–15 h). The reaction mixture was concentrated in vacuo. Chromatography (SiO_2, 3 cm × 15 cm, ethyl acetate eluant) afforded **16f** (120 mg, 246 mg theoretical, 49%) as a pale yellow solid: mp 160–162 °C dec (EtOAc–EtOH, 1:1, white platelets); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.14 (s, 1 H), 4.23 (t, 2 H, *J* = 6 Hz), 4.11 (s, 3 He, 3.04 (t, 2 H, *J* = 6 Hz), 2.26 (p, 2 H, *J* = 6 Hz); IR (KBr) ν_{max} 3060, 2954, 1744, 1580, 1442, 1352, 1201, 1143, 1091, 952, 822, 742 cm^{-1}; EIMS, *m/e* (relative intensity) 222 (M^+, 6), 192 (4), 164 (59), 152 (10), 119 (11), 105 (20), 92 (base), 59 (31); CIMS (2-methylpropane), *m/e* 223 (M^+ + H, base); HRMS, *m/e* 222.0645 ($C_{10}H_{10}N_2O_4$ requires 222.0638).

Dimethyl 4-[1-((*tert*-Butyldimethylsilyl)oxy)methyl]-5-ethyl-1,2-diazine-3,6-dicarboxylate (16g). Following the procedure for the preparation of **16a** [1-[(*tert*-butyldimethylsilyl)-oxy]-2-pentyne (66 μL, 0.30 mmol)[12] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 50 mg, 0.25 mmol);[11] Table II] provided 50 mg (92 mg theoretical, 54%) of **16g**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 4.82 (s, 2 H), 3.95 (s, 3 H), 3.92 (s, 3 H), 2.79 (q, 2 H, *J* = 7 Hz), 1.15 (t, 3 H, *J* = 7 Hz), 0.78 (s, 9 H), 0.05 (s, 6 H); IR (film) ν_{max} 2955, 2886, 1742, 1541, 1440, 1391, 1277, 1198, 1076, 1036, 1007, 963, 821, 781 cm^{-1}; EIMS, *m/e* (relative intensity) 369 (M^+ + 1, 2), 353 (1), 337 (5), 311 (base), 267 (10), 235 (14), 191 (5), 162 (4), 133 (11), 89 (92), 73 (31), 59 (57); CIMS (2-methylpropane), *m/e* 369 (M^+ + H, base); HRMS, *m/e* 368.1765 ($C_{17}H_{28}N_2O_5Si$ requires 368.1767).

5,7-Dihydro-3-(methoxycarbonyl)-4-ethyl-7-oxofuro[3,4-*c*]pyridazine (16h). Following the procedure for the preparation of **16a** [2-pentyn-1-ol (0.28 mL, 3.08 mmol)[12] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**7**, 510 mg, 2.57 mmol);[11] Table II] provided 262 mg (570 mg theoretical, 46%) of **16h**: mp 118–119 °C (EtOAc, pale yellow needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 5.49 (s, 2 H), 4.12 (s, 3 H), 2.98 (q, 2 H, *J* = 7 Hz), 1.35 (t, 3 H, *J* = 7 Hz); IR (KBr) ν_{max} 2955, 1798, 1722, 1596, 1448, 1379, 1292, 1250, 1131, 1099, 1026, 994, 926, 818, 777 cm^{-1}; EIMS, *m/e* (relative intensity) 222 (M^+, 1), 196 (5), 164 (22), 138 (64), 121 (5), 105 (4), 93 (6), 79 (base), 59 (25); CIMS (2-methylpropane), *m/e* 223 (M^+ + H, base); HRMS, 222.0643 ($C_{10}H_{10}N_2O_4$ requires 222.0641).

Anal. Calcd for $C_{10}H_{10}N_2O_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 53.83; H, 4.57; N, 12.89.

Dimethyl 4-Vinyl-1,2-diazine-3,6-dicarboxylate (16i). Following the procedure for the preparation of **16a** [1-(benzyloxy)-3-butyne (0.53 mL, 3.0 mmol)[38] and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (7, 500 mg, 2.5 mmol);[11] Table II] provided 330 mg (555 mg theoretical, 60%) of **16i**: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.36 (s, 1 H), 7.25 (dd, 1 H, *J* = 11, 17 Hz), 6.15 (d, 1 H, *J* = 17 Hz), 5.82 (d, 1 H, *J* = 11 Hz), 4.11 (s, 3 H), 4.08 (s, 3 H); IR (film) ν_{max} 3007, 2956, 2852, 1731, 1619, 1579, 1446, 1390, 1263, 1137, 1058, 981, 959, 826, 738, 707 cm^{-1}; EIMS, *m/e* (relative intensity) 222 (M^+, 5), 192 (10), 164 (20), 122 (6), 105 (86), 91 (12), 77 (28), 59 (base); CIMS (2-methylpropane), *m/e* 223 (M^+, + H, base); HRMS, *m/e* 222.0648 ($C_{10}H_{10}N_2O_4$ requires 222.0641).

1,4,4a,6,7,8a-Hexahydro-3,8a-bis(methoxycarbonyl)-5*H*-pyrano[2,3-*c*]pyridazine (16j). Following the procedure for the preparation of **16a** [4-penten-1-ol (0.57 mL, 6.18 mmol)[12] and-dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (7, 1.02 g, 5.15 mmol);[11] Table II] provided 1.21 g (1.32 g theoretical, 92%) of **16j**: mp 104–105 °C (hexane, white needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 6.64 (br s, 1 H), 3.95 (m, 2 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 2.59 (m, 1 H), 2.52 (m, 1 H), 2.40 (m, 2 H), 1.64 (m, 2 H), 1.32 (m, 1 H); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm) 169.3 (s), 164.6 (s), 131.9 (s), 82.8 (s), 62.1 (t), 52.8 (q), 51.9 (q), 28.1 (d), 27.0 (t), 25.6 (t), 23.6 (t); IR (KBr) ν_{max} 3328, 2956, 1754, 1707, 1606, 1453, 1374, 1303, 1264, 1187, 1156, 1091, 1070, 995, 858, 656, 613 cm^{-1}; EIMS, *m/e* (relative intensity) 256 (M^+, 0.5), 225 (1), 197 (base), 165 (6), 155 (4), 137 (2), 123 (3), 111 (3), 95 (3), 79 (5); CIMS (2-methylpropane), *m/e* 256 (M^+ + H, base); HRMS, *m/e* 256.1055 ($C_{11}H_{16}N2O_5$ requires 256.1060).

Dimethyl 3-(3-Hydroxy-1-propyl)pyrrole-2,5-dicarboxylate (17e). Zinc dust (318 mg, 4.9 mmol, 10 equiv) was added to a solution of 1,2-diazine **16e** (124 mg, 0.49 mmol) in acetic acid (6 mL) at 25 °C, and the reaction mixture was stirred at 25 °C for 18 h. The reaction mixture was filtered through Celite, and the filtrate was made basic with the addition of 10% aqueous ammonium hydroxide. The aqueous phase was extracted with ethyl acetate (3 × 25 mL), and the combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 3 cm × 10 cm, ether eluant) afforded **17e** (50 mg, 118 mg theoretical, 42%) as a yellow solid: mp 99–100 °C (hexane–EtOAc, 1:1, white platelets); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 9.60 (br s, 1 H), 6.78 (d, 1 H, *J* = 2 Hz), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.81 (t, 2 *H*, *J* = 7 Hz), 2.86 (t, 2 H, *J* = 7 Hz), 2.20 (s, 1 H, OH), 1.84 (p, 2 H, *J* = 7 Hz); IR (KBr) ν_{max} 3278, 3009, 2951, 2917, 1863, 1730, 1562, 1475, 1441, 1267, 1090, 1012, 951, 835, 633 cm^{-1}; EIMS, *m/e* (relative intensity) 241 (M^+, 23), 223 (4), 210 (8), 197 (67), 164 (base), 146 (46), 138 (88), 120 (13), 106 (31), 91 (2), 77 (16); CIMS (2-methylpropane), *m/e* 242 (M^+ + H, base); HRMS, *m/e* 241.0945 ($C_{11}H_{15}NO_5$ requires 241.0950).

Anal. Calcd for $C_{11}H_{15}NO_5$: C, 54.77; H, 6.22; N, 5.81. Found: C, 54.40; H, 6.29; N, 6.00.

1,4,5,7-Dihydro-3-(methoxycarbonyl)-5-*n*-butyl-7-oxofuro[3,4-*c*]dihydropyridazine (18a). Zinc dust (728 mg, 11.2 mmol, 10.0 equiv) was added to a solution of lactone **16a** (280 mg, 1.12 mmol) in trifluoroacetic acid (2 mL) and the reaction mixture was stirred at 25 °C for 18 h. The reaction mixture was filtered through Celite and the filtrate made basic with saturated sodium bicarbonate. The aqueous phase was extracted with ether (2 × 50 ml), and the combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 3 cm × 10 cm, 75% ether–hexane eluant) afforded **18a** (211 mg, 282 mg, theoretical, 75%) as a yellow semisolid: ^1H NMR ($CDCl_3$, 80 MHz, ppm) 7.95 (br s, 1 H), 4.90 (m, 1 H), 3.85 (s, 3 H), 3.45 (d, 2 H, *J* = 2 Hz), 1.60–1.25 (m, 6 H), 0.90 (m, 3 H); IR (KBr) ν_{max} 3314, 1761, 1701, 1545, 1339, 1265, 1051, 985, 880, 810 cm^{-1}; EIMS, *m/e* (relative intensity) 252 (M^+, 32), 229 (3), 209 (base), 195 (18), 174 (10), 137 (16), 120 (20), 106 (25), 71 (24); HRMS, *m/e* 252.1084 ($C_{12}H_{16}O_4N_2$ requires 252.1111).

4,7-Dihydro-2-(methoxycarbonyl)-7-oxopyrano[3,4-*b*]-pyrrole (17c). Following the procedure for the preparation of **17e**, lactone **16c** (55 mg, 0.26 mmol) provided 15 mg (51 mg theoretical, 30%) of **17c**: mp 178–179 °C (hexane, white needles); ^1H NMR ($CDCl_3$, 200 MHz, ppm) 8.36 (s, 1 H), 6.73 (d, 1 H, *J* = 2 Hz), 4.57 (t, 2 H, *J* = 6 Hz), 3.91 (s, 3 H), 2.92 (t, 2 H, *J* = 6 Hz); IR (KBr) ν_{max} 3294, 1725, 1699, 1564, 1485, 1426, 1321, 1264, 1104, 1077, 1022, 997, 934, 779, 610 cm^{-1}; EIMS *m/e* (relative intensity) 195 (M^+, base), 165 (59), 146 (21), 137 (64), 122 (13),

(38) Johnson, W. S.; Wiedhaup, K.; Brady, S. F.; Olson, G. L. *J. Am. Chem. Soc.* **1974**, *96*, 3797.

165 (22), 79 (22), 51 (22); CIMS (2-methylpropane), m/e 196 ($M^+ + H$, base); HRMS, m/e 195.0533 ($C_9H_9NO_4$ requires 195.0532).

4,5,6,8-Tetrahydro-2-methoxycarbonyl-8-oxooxepino[3,4-*b*]pyrrole (17f). Following the procedure for the preparation of **17e**, lactone **16f** (113 mg, 0.51 mmol) provided 53 mg (107 mg theoretical, 50%) of **17f**: ^1H NMR ($CDCl_3$ 80 MHz, ppm) 9.68 (br s, 1 H), 6.75 (d, 1 H, J = 2 Hz), 4.38 (m, 2 H, J = 7 Hz), 3.81 (s, 3 H), 2.25 (t, 2 H, J = 7 Hz), 2.13 (m, 2 H); IR (film) ν_{max} 3287, 2963, 1718, 1560, 1475, 1439, 1262, 1101, 799, 686, 608 cm^{-1}; EIMS m/e (relative intensity) 209 (M^+, base), 197 (38), 178 (20), 164 (55), 153 (36e, 146 (27), 138 (70), 125 (38), 106 (24), 91 (14), 77 (15); HRMS, m/e 209.0686 ($C_{10}H_{11}NO_4$ requires 209.0688).

4,6-Dihydro-2-(methoxycarbonyl)-3-ethyl-6-oxofuro[3,4-*b*]pyrrole (17h). Following the procedure for the preparation of **17e**, lactone **16h** (60 mg, 0.27 mmol), provided 18 mg (56 mg theoretical, 32%) of **17h**: mp 182–184 °C (EtOAc, white needles); ^1H NMR ($CDCl_3$, 80 MHz, ppm) 9.65 (br s, 1 H), 5.20 (s, 2 H), 3.95 (s, 3 H), 2.85 (q, 2 H, J = 7 Hz), 1.25 (t, 3 H, J = 7 Hz); IR (KBr) ν_{max} 3233, 2925, 1740, 1701, 1557, 1469, 1386, 1274, 1193, 1032, 971, 843, 734 cm^{-1}; EIMS, m/e (relative intensity) 209 (M^+, base), 194 (99), 176 (86), 162 (25), 148 (39), 134 (17), 120 (10), 92 (5), 77 (16); CIMS (2-methylpropane), m/e 210 $M^+ + H$, base); HRMS, m/e 209.0687 ($C_{10}H_{11}NO_4$ requires 209.0688).

4-Methoxy-5-(methoxycarbonyl)-2,2′-bipyrrole (20a). Lithium methoxide in methanol (0.12 mL of 1.0 M, 0.12 mmol) was added to a solution of **15a** (30 mg, 0.12 mmol) in methanol (1 mL) at 25 °C, and the reaction mixture was stirred at 25 °C for 5 min. The reaction mixture was poured onto water (3 mL) and extracted with ethyl acetate (6 × 10 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 1 cm × 5 cm, ethyl acetate eluant) afforded **20a** (23 mg, 26 mg theoretical, 88%) as a gray solid: mp 206–208 °C (methanol, platelets); ^1H NMR (CD_3COCD_3, 200 MHz, ppm) 6.98 (m, 1 H), 6.64 (m, 1 H), 6.22 (s, 1 H), 6.19 (m, 1 H), 3.80 (s, 3 H), 3.72 (s, 3 H); IR (KBr) ν_{max} 3317, 3002, 2951, 1642 (CO_2CH_3), 1556, 1501, 1463, 1348, 1303, 1234, 1194, 1116, 1022, 965, 888, 784, 763 cm^{-1}; CIMS (2-methylpropane), m/e 221 ($M^+ + H$, base); HRMS, m/e 220.0854 ($C_{11}H_{12}N_2O_3$ requires 220.0848).

5-(Methoxycarbonyl)-2,2′-bipyrrole (20b). Lithium methoxide in methanol (1.15 mL of 1.0 M, 1.15 mmol) was added to a solution of **15b** (250 mg, 1.15 mmol) in methanol (3.4 mL) at 25 °C, and the reaction mixture was stirred at 25 °C for 5 min. The reaction mixture was poured onto water (5 mL) and extracted with ethyl acetate (6 × 25 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (SiO_2, 2 cm × 10 cm, 50% ether–hexane eluant) afforded **20b** (208 mg, 219 mg theoretical, 95%) as a gray solid: mp 216–217 °C dec (EtOAc–hexane, platelets); ^1H NMR (CD_3COCD_3, 200 MHz, ppm) 10.82 (br s, 1 H), 10.51 (br s, 1 H), 6.86 (m, 2 H), 6.84 (m, 1 H), 6.44 (m, 1 H), 6.16 (m, 1 H), 3.76 (s, 3 H); ^{13}C NMR (CH_3CN, 50 MHz, ppm) 161.7 (s), 132.6 (s), 125.5 (s), 122.2 (s), 119.8 (d), 117.3 (d), 109.9 (d), 106.8 (d), 105.9 (d), 51.2 (q); IR (KBr) ν_{max} 3330, 3220, 2940, 1670, 1490, 1450, 1350, 1290, 1260, 1190, 1160, 1120, 1050, 1000, 790, 750, 710 cm^{-1}; EIMS, m/e (relative intensity) 190 (M^+, 96), 158 (74), 130 (base), 111 (2), 104 (21), 94 (6), 79 (8); CIMS (2-methylpropane), m/e 191 ($M^+ + H$, base); HRMS, m/e 190.0730 ($C_{10}H_{10}N_2O_2$ requires 190.0742).

4-Methoxy-2,2′-bipyrrole-5-carboxaldehyde (21a). Following the procedure detailed by Rapoport and Wasserman,[1c,d] a solution of ester **20a** (25 mg, 0.113 mmol) in anhydrous hydrazine (1 mL) was stirred at 25 °C for 4 h. The reaction mixture was concentrated in vacuo to afford the corresponding acyl hydrazine (24.5 mg, 25.0 mg theoretical, 98%). A solution of the crude hydrazide (24.5 mg, 0.111 mmol) in pyridine (1 mL) was treated with *p*-toluenesulfonyl chloride (21 mg, 0.111 mmol) at 25 °C, and the reaction mixture was allowed to stir at 25 °C for 15 min. The reaction mixture was poured onto water (1 mL) and the resulting precipitate was collected by filtration to afford the *p*-toluenesulfonyl hydrazide (40 mg, 41 mg theoretical, 97%). Sodium carbonate (48 mg, 0.45 mmol) was added to a slurry of the *p*-toluenesulfonyl hydrazide (50 mg, 0.13 mmol) in ethylene glycol (0.3 mL), and the solution was warmed at 170 °C for 5 min. The reaction mixture was cooled to room temperature, poured onto water (75 mL), and extracted with ether (5 × 25 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (neutral alumina, activity grade III, ethyl acetate eluant) afforded **21a** (8.5 mg, 25 mg theoretical, 34%):[37] ^1H NMR ($CDCl_3$, 200 MHz, ppm) 9.19 (s, 1 H), 6.94 (m, 1 H), 6.64 (m, 1 H), 6.26 (m, 1 H), 5.99 (s, 1 H), 3.90 (s, 3 H); EIMS, m/e (relative intensity), 190 (M^+, base), 172 (6), 161 (6), 145 (67), 129 (6), 108 (11), 97 (6), 91 (33), 71 (14); CIMS (2-methylpropane), m/e 191 ($M^+ + H$, base); HRMS, m/e 190.0760 ($C_{10}H_{10}N_2O_2$ requires 190.0742).

2,2′-Bipyrrole-5-carboxaldehyde (21b). Following the procedure for the preparation of **21a**, a solution of ester **20b** (100 mg, 0.52 mmol) in anhydrous hydrazine (3 mL) was stirred at 25 °C for 4 h. The reaction mixture was concentrated in vacuo to give the acyl hydrazide (100 mg, 100 mg theoretical, 100%). A solution of the crude hydrazine (100 mg, 0.52 mmol) in pyridine (3 mL) at 25 °C was treated with *p*-toluenesulfonyl chloride (100 mg, 0.52 mmol) and the reaction mixture was allowed to stir at 25 °C for 15 min. The reaction mixture was poured onto water (3 mL), and the resulting precipitate was collected by filtration to give the *p*-toluenesulfonyl hydrazide (177 mg, 179 mg theoretical, 98%). Sodium carbonate (46 mg, 0.43 mmol) was added to a slurry of the *p*-toluenesulfonyl hydrazide (50 mg, 0.14 mmol) in ethylene glycol (0.3 mL) at 25 °C, and the solution was warmed at 170 °C for 5 min. The reaction mixture was cooled to room temperature, poured onto water (75 mL), and extracted with ether (5 × 25 mL). The combined ether extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatography (neutral alumina, activity grade III, 2 cm × 5 cm, ethyl acetate eluant) afforded **21b** (8.6 mg, 22 mg theoretical, 39%)[37] as a yellow solid: ^1H NMR (CD_3CN, 200 MHz, ppm) 9.41 (s, 1 H), 7.05 (m, 1 H), 6.85 (m, 1 H), 6.70 (m, 1 H), 6.45 (m, 1 H), 6.15 (m, 1 H); IR (KBr) ν_{max} 3500, 2910, 2830, 1630, 1600, 1490, 1290, 1050 cm^{-1}; EIMS, m/e (relative intensity) 160 (M^+, base), 132 (11), 131 (43), 105 (12), 104 (44), 91 (7), 77 (10), 57 (10); CIMS (2-methylpropane), m/e 161 ($M^+ + H$, base); HRMS, m/e 160.0629 ($C_9H_8N_2O$ requires 160.0637).

Prodigiosin (1). 2-Methyl-3-pentylpyrrole (1.81 mg, 0.012 mmol) was added to a solution of aldehyde **21a** (2.31 mg, 0.012 mmol) in methanol (0.5 mL) at 25 °C, and the resulting solution was warmed at 100 °C for 5 min. While still warm concentrated hydrochloric acid (one drop) was added and the reaction mixture was allowed to stand at 25 °C for 30 min. The reaction mixture was concentrated in vacuo. Chromatography (neutral alumina, activity grade III, 2 cm × 10 cm, ether eluant) afforded **1** (2.3 mg, 3.9 mg theoretical, 59%) as an orange solid: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 6.84 (s, 1 H), 6.74 (m, 2 H), 6.30 (s, 1 H), 6.12 (t, 1 H, J = 3 Hz), 6.08 (s, 1 H), 3.96 (s, 3 H), 2.26 (t, 2 H, J = 8 Hz), 1.68 (s, 3 H), 1.46 (m, 2 H), 1.28 (m, 4 H), 0.88 (t, 3 H, J = 7 Hz); IR (KBr) ν_{max} 3424, 3105, 2955, 1624, 1579, 1465, 1361, 1280, 1232, 1188, 1149, 1119, 1060, 1032, 1000, 959, 892, 806, 770, 732, 651 cm^{-1}; EIMS, m/e (relative intensity) 323 (M^+, base), 266 (61), 250 (3), 227 (1), 162 (2), 133 (2), 91 (4); CIMS (2-methylpropane), m/e 324 ($M^+ + H$, base); HRMS, m/e 323.2017 ($C_{20}H_{25}N_3O$ requires 323.1998).

The free base of **1** in $CHCl_3$ was converted to its HCl salt ($CHCl_3$ soluble) by treatment with 10% aqueous HCl. **1**·HCl: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.25 (m, 1 H), 6.97 (s, 1 H), 6.94 (m, 1 H), 6.69 (d, 1 H, J = 2.5 Hz), 6.37 (m, 1 H), 6.09 (d, 1 H, J = 2 Hz), 4.01 (s, 3 H), 2.55 (s, 3 H), 2.4 (t, 2 H, J = 7 Hz), 1.55 (m, 2 H), 1.34 (m, 4 H), 0.90 (t, 3 H, J = 7 Hz).

Prodigiosene (2a). Pyrrole (2.4 μL, 0.035 mmol) was added to a solution of aldehyde **21b** (5.6 mg, 0.035 mmol) in methanol (0.5 mL) at 25 °C, and the solution was warmed at 100 °C for 5 min. While still warm, concentrated hydrobromic acid (three drops) was added, and the reaction mixture was allowed to stand at 25 °C for 30 min. The reaction mixture was concentrated in vacuo. Chromatography (neutral alumina, activity grade III, 2 cm × 10 cm, ether eluant) afforded **2a** as the free base. Prodigiosene (**2a**) in $CHCl_3$ was immediately converted to the HBr salt ($CHCl_3$ soluble) by treatment with 48% aqueous HBr to afford **2a**·HBr (3.6 mg, 10.1 mg theoretical, 36%) as a purple solid: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.68 (m, 1 H), 7.40 (m, 1 H), 7.22 (m, 1 H), 7.17 (m, 1 H), 7.13 (s, 1 H), 7.10 (m, 1 H), 6.95 (m, 1 H), 6.55 (m, 1 H), 6.45 (m, 1 H); IR (KBr) ν_{max} 3852, 3397, 3158, 2924, 1600, 1514, 1458, 1366, 1294, 1125, 958, 795, 699 cm^{-1}; UV (95% EtOH, HCl) λ_{max} 546 nm;[1b] EIMS, m/e (relative intensity)

209 (M^+, 20), 167 (2), 153 (3), 137 (3), 133 (5), 109 (6), 82 (25), 67 (base); CIMS (2-methylpropane), m/e 210 ($M^+ + H$, 17); HRMS, m/e 209.0943 ($C_{13}H_{11}N_3$ requires 209.0953).

2-Methyl-3-pentylprodigiosene (Desmethoxyprodigiosin, 2e). 2-Methyl-3-pentylpyrrole (4.4 mg, 0.029 mmol) was added to a solution of the aldehyde **21b** (4.7 mg, 0.029 mmol) in methanol (0.5 mL) at 25 °C, and the solution was warmed at 100 °C for 5 min. While still warm, concentrated hydrobromic acid (three drops) was added to the solution, and the reaction mixture was allowed to stand for 30 min (25 °C). The reaction mixture was concentrated in vacuo. Chromatography (neutral alumina, activity grade III, 2 cm × 10 cm, ether eluant) afforded **2e** as the free base. 2-Methyl-3-pentylprodigiosene (**2e**) in $CHCl_3$ was immediately converted to the HBr salt ($CHCl_3$ soluble) by treatment with 48% aqueous HBr to afford **2e**·HBr (4.8 mg, 10.8 mg theoretical, 44%) as a purple solid: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 7.6 (m, 1 H), 7.15 (m, 1 H), 6.96 (m, 1 H), 6.88 (s, 1 H), 6.8 (m, 1 H), 6.58 (m, 1 H), 6.27 (m, 1 H), 2.62 (s, 3 H), 2.40 (t, 2 H, J = 7 Hz), 1.64 (m, 2 H), 1.35 (m, 4 H), 0.92 (t, 3, H, J = 7 Hz) [accompanied by the free base (or E isomer) [2.76 (t, 2H, J = 7 Hz), 2.56 (s, 3 H), 1.58 (m, 2 H), 0.90 (t, 3 H, J = 7 Hz)]: IR (KBr) ν_{max} 3745, 3451, 2925, 1635, 1602, 1561, 1510, 1278, 1129, 1059, 962, 789, 717 cm^{-1}; UV (95% EtOH, HCl) λ_{max} 568 nm;[1b] EIMS, m/e (relative intensity), 293 (M^+, 60), 237 (18), 236 (base), 193 (5), 151 (8), 108 (4), 94 (54), 80 (8); CIMS (2-methylpropane), m/e 294 ($M^+ + H$, 44); HRMS, m/e 293.1900 ($C_{19}H_{23}N_3$ requires 293.1892).

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Registry No. 1, 82-89-3; 1·HCl, 112373-40-7; **2a**, 22187-69-5; **2a**·HBr, 22187-70-8; **2e**, 112373-41-8; **2e**·HBr, 22187-75-3; **7**, 2166-14-5; **8**, 92144-07-5; **9**, 92144-13-3; **10**, 112373-15-6; **11**, 112373-16-7; **12a**, 112373-17-8; **12b**, 1193-62-0; **13a**, 21972-99-6; **13b**, 107962-24-3; **13c**, 92776-70-0; **14a**, 112373-18-9; **14b**, 107962-26-5; **14c**, 107962-25-4; **15a**, 112373-19-0; **15b**, 112373-20-3; **16a**, 112373-21-4; **16b**, 112373-22-5; **16c**, 112373-23-6; **16d**, 112373-24-7; **16e**, 23900-45-0; **16f**, 112373-25-8; **16g**, 112373-27-0; **16h**, 112373-28-1; **16i**, 112373-29-2; **16j**, 112373-30-5; **17c**, 112373-33-8; **17e**, 112373-31-6; **17f**, 112373-34-9; **17h**, 112373-35-0; **18a**, 112373-32-7; **18b**, 112373-42-9; **18c**, 112373-43-0; **20a**, 112373-36-1; **20a** (hydrazide), 112373-37-2; **20a** ((*p*-toluenesulfonyl)hydrazide), 112373-38-3; **20b**, 106480-92-6; **20b** (hydrazide), 112373-44-1; **20b** ((*p*-toluenesulfonyl)hydrazide), 112373-39-4; **21a**, 10476-41-2; **21b**, 22187-87-7; $CH_2{=}C(OCH_3)_2$, 922-69-0; $CH_3(CH_2)_3CH(OH)C{\equiv}CH$, 7383-19-9; $CH_3(CH_2)_3C(O)C{\equiv}CH$, 26119-02-8; $HOCH_2CH_2C{\equiv}CH$, 927-74-2; *t*-$BuSi(Me)_2O(CH_2)_2C{\equiv}CH$, 78592-82-2; $HOCH_2(CH_2)_2C{\equiv}CH$, 5390-04-5; *t*-$BuSi(Me)_2OCH_2C{\equiv}CEt$, 112373-26-9; $HOCH_2C{\equiv}CEt$, 6261-22-9; $PhCH_2O(CH_2)_2C{\equiv}CH$, 22273-77-4; $HO(CH_2)_3CH{=}CH_2$, 821-09-0; 2-methyl-3-pentylpyrrole, 18320-91-7; pyrrole, 109-97-7; 5-(ethoxycarbonyl)-2-methyl-3-valeroylpyrrole, 92198-32-8.

Supplementary Material Available: Summary Table and summary figure of the INDO and AMPAC, AM1, treatment of prodigiosin and 2-methyl-3-pentylprodigiosene (2 pages). Ordering information is given on any current masthead page. Optimized MM2 (MacroModel, version 1.1) geometries and total energy for linear, nonlinear (*E*/*Z*)-**1**, -**2a**, and -**2e** (4 pages), and the final results (bond lengths, bond angles, cartesian coordinates, interatomic distances, eigenvectors, net atomic charges and dipole contributions, atomic orbital electron populations, state energies, and C.I.) of the AMPAC, AM1 (version 1.0) optimization/SCF calculation for prodigiosin (**1**) and 2-methyl-3-pentylprodigiosene (**2e**) are available from D.L.B. upon request.

Inverse Electron Demand Diels–Alder Reactions of 3,6-Bis(methylthio)-1,2,4,5-tetrazine: 1,2-Diazine Introduction and Direct Implementation of a Divergent 1,2,4,5-Tetrazine → 1,2-Diazine → Benzene (Indoline/Indole) Diels–Alder Strategy†

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A full investigation of the scope of the participation of 3,6-bis(methylthio)-1,2,4,5-tetrazine (**6**) in [4 + 2] cycloaddition reactions is detailed. The use of the resulting 3,6-bis(methylthio)-1,2-diazine cycloadducts as direct precursors to the parent 4,5-substituted-1,2-diazines as well as alkyne/allene 1,2-diazines suitable for use in subsequent intramolecular Diels–Alder reactions is described. The latter application constitutes the direct implementation of a divergent 1,2,4,5-tetrazine → 1,2-diazine → benzene (indoline/indole) Diels–Alder strategy.

In recent efforts we have detailed the use of a series of inverse electron demand Diels–Alder reactions of electron-deficient heterocyclic azadienes[2–11] in [4 + 2] cycloaddition reactions with electron-rich dienophiles comprising a general approach to the introduction of a range of heteroaromatic systems, Scheme I. The approach has been proven to be well-suited for the preparation of highly substituted and highly functionalized heteroaromatic systems difficult to assemble by alternative methodology

† This paper is dedicated to Professor E. C. Taylor on the occasion of his 65th birthday.

(1) National Institutes of Health research career development award recipient, 1983–88 (CA 01134). Alfred P. Sloan research fellow, 1985–89.
(2) Boger, D. L. *Tetrahedron* **1983**, *39*, 2869. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781.
(3) Boger, D. L.; Panek, J. S. *J. Org. Chem.* **1981**, *46*, 2179. Boger, D. L.; Panek, J. S. *J. Org. Chem.* **1982**, *47*, 895.
(4) Boger, D. L.; Panek, J. S. *J. Org. Chem.* **1982**, *47*, 3763. Boger, D. L.; Panek, J. S. *Tetrahedron Lett.* **1984**, *25*, 3175. Boger, D. L.; Duff, S. R.; Panek, J. S.; Yasuda, M. *J. Org. Chem.* **1985**, *50*, 5782.

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