Synthesis of the Pyrazolo[4,3-*e*][1,2,4]triazine Family of Natural Products: Nostocine A, Fluvioi A and Pseudoiodinine

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General Information

¹H- and ¹³C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer, Varian Gemini 400 MHz spectrometer or a Varian Unity INOVA 500 MHz spectrometer. The chemical shifts are reported relative to tetramethylsilane (0.00 ppm) as the internal standard with the exception of spectra recorded in dichloromethane-d₂ (DCM-d₂), which used the solvent residual peak (5.32 ppm). NOe difference, NOESY2D, HMBC and HMQC spectra were recorded on a Varian Unity INOVA 500 MHz spectrometer using pulse field gradients. High-resolution mass spectra were obtained from the University of Illinois, Urbana-Champaign spectrometry facility. Infrared spectra were recorded using a Nicolet Avatar 360 FT-IR. UV-Vis spectra were recorded on an Agilent 8453 spectrometer. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected.

Acetonitrile (CH₃CN), diethyl ether (Et₂O) and dichloromethane (DCM) were dried by passage through a column of activated alumina under a positive pressure of nitrogen.¹ Absolute ethanol (EtOH) and anhydrous methanol (MeOH) were purchased from Acros and used without further purification. Chloroform (CHCl₃) was purchased from Doe and Ingalls and contained 0.2 % EtOH as stabilizer. Ethyl acetate (EtOAc), hexanes, CHCl₃, MeOH, and DCM were distilled prior to use in chromatography. Phosphorus oxychloride (POCl₃) was distilled from sodium metal under an atmosphere of Ar and used within 4 weeks. All other reagents were purchased from either Aldrich or Acros and used as supplied. Unless otherwise noted, chromatographic separations were performed by standard flash chromatography techniques using reagent silica gel (60 Å) purchased from Bodman. Medium Pressure Liquid Chromatography (MPLC) was performed using a Biotage Horizon HPFC™ system with pre-packed KP-silica gel (60 Å) cartridges at 30 psi. Rotary evaporation was normally carried out at a pressure of ca 6 torr using a diaphragm vacuum pump.

Experimental Procedures

Diethyl 2-((Dimethylamino)methyleneamino)malonate (**5**).²

In a flame-dried, 1 L round-bottom flask under an atmosphere of Ar, thin strips of pressed sodium metal (5.65 g, 246 mmol) were added to ice-cold absolute EtOH (300 mL) over ca 5 min. After the sodium was consumed (ca 30 min), diethyl aminomalonate hydrochloride (51.1 g, 246 mmol) was added in one portion. The flask was removed from the ice bath and stirred at ambient temperature for 15 min. The volatiles were then removed by rotary evaporation and the crude residue was diluted with benzene (250 mL). After addition of *N,N*-dimethylformamide dimethyl acetal (45.0 mL, 317 mmol), the cloudy solution was heated at reflux for 15 min. After standing at ambient temperature overnight, the resulting precipitate (NaCl) was removed by filtration through a plug of packed Celite. Removal of the benzene by rotary evaporation, followed by addition of 1:1 (v/v) EtOAc:hexanes (100 mL) and subsequent evaporation yielded **5** as an off-white solid (50.6 g, 89%). Mp 26–30 °C. ¹H NMR (300 MHz, CDCl₃, δ): 1.28 (t, *J* = 7.2 Hz, 6H), 2.93 (s, 6H), 4.23 (q, *J* = 7.2 Hz, 4H), 4.47 (s, 1H), 7.44 (s, 1H).

Ethyl 1,4,5,6-Tetrahydro-6-oxo-1,2,4-triazine-5-carboxylate (**6**).³

In a flame-dried, 2-neck round-bottom flask under an atmosphere of Ar, amidine **5** (47.0 g, 204 mmol) was stirred in absolute EtOH (300 mL). The suspension was heated to reflux (all of the amidine was now dissolved). To the refluxing solution was added a solution of anhydrous hydrazine (8.20 mL, 260 mmol) in absolute EtOH (300 mL) dropwise via an addition funnel over ca 1 h. The reaction was heated at reflux for an additional 30 min and then cooled in an ice-water bath for 1 h. Crude 5-hydrazinocarbonyl-1,2,5,6-tetrahydro-6-oxo-1,2,4-triazine^{2a} (7.4 g, 23%) was removed by filtration. The filtrate was evaporated to dryness and the resulting oily orange solid was mostly dissolved in 1:4 (v/v) MeOH: EtOAc

¹ Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

² a) Smodiš, J.; Zupet, R.; Petrič, A.; Stanovnik, B.; Tišler, M. *Heterocycles* **1990**, *30*, 393–405. b) The procedure to make **5** was adapted slightly from the one presented in the paper.

³ Procedure adapted from the synthesis of 5-hydrazinocarbonyl-1,2,5,6-tetrahydro-6-oxo-1,2,4-triazine reported in ref. 2a.

(750 mL). The suspension was filtered through a plug of Al₂O₃ (Brockmann III, 100 g) and the plug was washed with 1:9 (v/v) MeOH: EtOAc (1 L). Rotary evaporation of the combined filtrate and wash yielded an oily yellow solid (24.0 g). The crude material was left to stand with EtOAc (400 mL) for 1 h and the resulting off-white precipitate was collected by filtration to afford **6** (15.1 g, 43%). Mp⁴ 128–131 °C. ¹H NMR (400 MHz, DMSO-d₆, δ): 1.19 (t, *J* = 7.2 Hz, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.54 (d, *J* = 1.6 Hz, 1H), 6.96 (d, *J* = 4 Hz, 1H), 7.55 (bs, 1H), 10.4 (bs, 1H). ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.0, 56.2, 61.3, 136.6, 156.5, 168.4. IR (KBr) (cm⁻¹): 3340, 3192, 3051, 2924, 1748, 1672, 1646, 1505. UV (EtOH) λ_{max} (log ε): 223 (3.3), 278 (3.3). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₆H₁₀N₃O₃, 172.0722; found, 172.0721.

Ethyl 1,6-Dihydro-6-oxo-1,2,4-triazine-5-carboxylate (**7**).

In a flame-dried, 1 L round-bottom flask under an atmosphere of Ar, dihydrotriazone **6** (15.1 g, 88.2 mmol) was dissolved in absolute EtOH (400 mL). Iodobenzene diacetate (29.3 g, 91.1 mmol) was added in one portion and the reaction was stirred at ambient temperature for 2 h. The volatiles were removed by rotary evaporation. The resulting oil was dissolved in CHCl₃ (25 mL) and purified by column chromatography using SiO₂ gel (4 x 16 cm) eluting first with 1:2 (v/v) EtOAc:hexanes until all of the iodobenzene was removed and then with 1:1 (v/v) EtOAc:hexanes, to yield **7** as a yellow powder (10.4 g, 70%). Mp 94–96 °C. ¹H NMR (400 MHz, CDCl₃, δ): 1.44 (t, *J* = 7.2 Hz, 3H), 4.51 (q, *J* = 7.2 Hz, 2H), 8.42 (s, 1H), 12.12 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃, δ): 13.9, 63.0, 141.4, 153.0, 157.9, 161.5. IR (KBr) (cm⁻¹): 3308, 3097, 3067, 2986, 1732, 1673, 1644, 1579, 1525. UV (EtOH) λ_{max} (log ε): 220 (3.7), 312 (3.4). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₆H₈N₃O₃, 170.0566; found, 170.0572.

Ethyl 6-Chloro-1,2,4-triazine-5-carboxylate (**8**).

In a flame-dried, 1 L round-bottom flask under an atmosphere of Ar, triazinone **7** (6.95 g, 41.1 mmol) and benzyltriethylammonium chloride (18.8 g, 82.5 mmol) were dissolved in anhydrous CH₃CN (165 mL). *N,N*-Dimethylaniline (6.25 mL, 49.3 mmol) was added, followed by addition of POCl₃ (19.0 mL, 204 mmol) in one portion. The flask was immediately lowered into an oil bath pre-heated to 120 °C. After 11 min (ca 6 min at reflux), the solution was cooled slightly and the volatiles were removed by rotary evaporation. After subsequent evacuation (ca 0.01 torr) for 15 min, the resulting black residue was then diluted with CHCl₃ (400 mL) and remaining POCl₃ was quenched by stirring with crushed ice for 15 min. The resulting layers were separated and the aqueous layer was extracted with additional CHCl₃ (3 x 50 mL). The organic layers were then combined, washed with saturated NaHCO₃ (1 x 150 mL) and evaporated. The resulting deep red liquid was dissolved in 1:3 (v/v) EtOAc:hexanes and rapidly passed through a short column of SiO₂ gel (6 x 7 cm). The resulting deep red-orange oil (5.89 g; contaminated with *N,N*-dimethylaniline) was typically used in subsequent steps without further purification; however, a pure sample could be obtained by MPLC purification of ca ¼ of the crude sample (1.56 g) using a Biotage 25+M SiO₂ gel column (40 g) eluting with EtOAc:hexanes(1:9→1:3, 510 mL linear gradient) to yield **8** as a yellow-orange oil (0.860 g, 45%). ¹H NMR (400 MHz, CDCl₃, δ): 1.47 (t, *J* = 7.2 Hz, 3H), 4.55 (q, *J* = 7.2 Hz, 2H), 9.72 (s, 1H). ¹³C NMR (100 MHz, CDCl₃, δ): 14.1, 63.8, 148.5, 153.9, 155.2, 161.3. IR (NaCl, neat) (cm⁻¹): 2984, 2939, 1743, 1513. UV (EtOH) λ_{max} (log ε): 274 (3.3), 388 (1.9). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₆H₇N₃O₂Cl, 188.0227; found, 188.0220.

N-(*tert*-Butoxycarbonyl)-*N*-methylhydrazine (**9**).⁵

In a flame-dried, 250 mL round-bottom flask under an atmosphere of Ar, di-*tert*-butyl dicarbonate (53.3 g, 245 mmol) was dissolved in anhydrous CH₃CN (135 mL) and the clear, colorless solution was cooled in an ice-water bath. Anhydrous methylhydrazine (10.0 mL, 184 mmol) was then added dropwise over 10 min and the solution was allowed to warm to ambient temperature. After 24 h, the solvent was removed by rotary evaporation and the crude product was fractionally distilled under vacuum (30–35 °C @ ca 0.1 torr; lit.⁵ 65 °C @ 10 torr) yielding **9** as a clear, colorless liquid (17.3 g, 64%). ¹H NMR (500 MHz, CDCl₃, δ): 1.47 (s, 9H), 3.06 (s, 3H), 4.09 (bs, 2H).

⁴ Attempts to crystallize **6** from mixtures of EtOAc:MeOH resulted in clear, colorless crystals. The crystals became opaque rapidly at 190–192 °C and melted sharply at 230–231 °C.

⁵ Procedure adapted from Metz, H. J.; Neunhoeffer, H. *Chem. Ber.* **1982**, *115*, 2807–2818.

Ethyl 6-(2-(*tert*-Butoxycarbonyl)-2-methylhydrazinyl)-1,2,4-triazine-5-carboxylate (10).

In a 500 mL round-bottom flask under an atmosphere of Ar, crude chlorotriazine **8** (1.75 g, from 2.53 g **7**) was dissolved in absolute EtOH (100 mL). Hydrazide **9** (2.76 g, 18.9 mmol) was added and the solution was heated to reflux. After 16 h at reflux, the reaction was cooled to ambient temperature and the solvent was removed by rotary evaporation. The crude residue was then dissolved in CHCl₃ (100 mL) and passed through a plug of SiO₂ gel (4 x 8 cm). The plug was washed with additional CHCl₃ (500 mL) and the combined filtrate and wash were evaporated to yield an orange oil (2.6 g). The crude product was purified by MPLC using a Biotage 40+M SiO₂ gel column (100 g) eluted with EtOAc:hexanes (1:3→1:1, 1320 mL linear gradient) to yield **10** as an orange oil (1.92 g, 43% in 2 steps from **7**). ¹H NMR (300 MHz, CDCl₃, δ): 1.37 (bs, 9H), 1.47 (t, *J* = 7.2 Hz, 3H), 3.34 (s, 3H), 4.52 (q, *J* = 7.2 Hz, 2H), 9.20 (bs, 1H), 9.40 (s, 1H). ¹³C NMR (100 MHz, CDCl₃, δ): 14.3, 28.4, 28.6, 38.4, 65.6, 130.4, 150.7, 155.2, 155.9, 165.4. IR (NaCl, neat) (cm⁻¹): 3351, 2930, 2930, 1712, 1556. UV (EtOH) λ_{max} (log ε): 241 (3.9), 353 (3.3). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₁₂H₂₀N₅O₄, 298.1515; found, 298.1511.

1,2-Dihydro-2-methyl-3*H*-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (11).⁶

In a 250 mL round-bottom flask, **10** (1.92 g, 6.46 mmol) was dissolved in a mixture of DCM (35 mL) and trifluoroacetic acid (35 mL). After 40 min at ambient temperature, the solvent was evaporated to constant weight and the resulting TFA salt was dissolved in THF (60 mL). The solution was cooled in an ice-water bath and NaH (974 mg, 60% dispersion in oil, 24 mmol) was added in one portion. After the initial evolution of gas had subsided, the reaction was removed from the ice bath and stirred at ambient temperature. After 40 min, the mixture was diluted with 1:1 (v/v) EtOAc:MeOH (35 mL) and passed through a plug of silica gel (4 x 8 cm). The plug was washed with additional 1:1 (v/v) EtOAc:MeOH (350 mL) and the filtrate was evaporated to yield a deep red solid. The crude material was then dissolved in MeOH (100 mL), evaporated onto SiO₂ (18.5 g) and purified by chromatography using SiO₂ (4 x 18 cm) eluting with a gradient from 1:3→1:1 EtOAc:MeOH to yield **11** as a red-purple solid (0.850 g, 87%). Mp 145 °C dec. ¹H NMR (300 MHz, CD₃OD, δ): 3.80 (s, 3H), 9.11 (s, 1H). ¹³C NMR (100 MHz, CD₃OD, δ): 38.8, 126.5, 147.6, 154.1, 157.3. IR (NaCl, thin film) (cm⁻¹): 3378, 1605, 1525. UV (EtOH) λ_{max} (log ε): 259 (4.0), 2.95(sh) (3.4), 503 (2.9). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₅H₆N₅O, 152.0572; found, 152.0565.

3-Methoxy-2-methyl-2*H*-pyrazolo[4,3-*e*][1,2,4]triazine (12).

In a flame-dried, 100 mL round-bottom flask under an atmosphere of Ar, **11** (0.0583 g, 0.384 mmol) was dissolved in a 9:1 (v/v) mixture of acetonitrile:MeOH (20 mL). The deep red solution was cooled in an ice-water bath and TMSCHN₂ (1.0 mL, 2.0 M, 2.0 mmol) was added dropwise over 1 min. After 45 min, the mixture was poured directly onto a plug of silica gel (2 x 4 cm). The plug was washed with 1:9 (v/v) MeOH:EtOAc (35 mL) and the eluate was evaporated to yield a mixture of dimethylated products as a green-purple oil (0.0430 g). The crude product was separated using a Biotage MPLC 12+M SiO₂ column (8 g) using EtOAc:hexanes (1:2→1:9, 120 mL linear gradient) to yield yellow **12** (0.0052 g, 8%) as well as orange 1,2-dihydro-1,2-dimethyl-3*H*-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (**22**; 0.0018 g, 3%) and purple 2,7-dihydro-2,7-dimethyl-3*H*-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (**23**; 0.0098 g, 15%).⁷

12: Mp⁸ 138–139 °C. ¹H NMR (400 MHz, CD₂Cl₂, δ): 4.09 (s, 3H), 4.60 (s, 3H), 9.50 (s, 1H). ¹³C NMR (100 MHz, CD₂Cl₂, δ): 26.7, 36.8, 60.6, 115.9, 149.7, 153.5. IR (NaCl, thin film) (cm⁻¹): 2921, 2851,

⁶ The absence of a carbonyl peak in the IR suggests that this molecule exists as a tautomer.

⁷ The structures of **22** and **23** were so assigned based on their synthesis from two different starting materials: **22** was isolated after the methylation of either **11** or **21** (see below); **23** was isolated after the methylation of either **2** (see below) or **11**.

1582. UV (EtOH) λ_{max} (log ϵ): 234 (4.1), 292 (3.7), 403 (3.2). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_6\text{H}_8\text{N}_5\text{O}$, 166.0729; found, 166.0736.

22: Mp⁸ 157–159°C. ¹H NMR (400 MHz, CD_2Cl_2 , δ): 3.58 (s, 3H), 3.68 (s, 3H), 9.72 (s, 1H). ¹³C NMR (100 MHz, CD_2Cl_2 , δ): 29.7, 35.0, 134.8, 154.1, 155.6, 157.5. IR (NaCl, thin film) (cm^{-1}): 2925, 1697, 1573. UV (EtOH) λ_{max} (log ϵ): 254 (4.1), 391 (3.3). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_6\text{H}_8\text{N}_5\text{O}$, 166.0729; found, 166.0726.

23: Mp 102–104°C. ¹H NMR (500 MHz, CD_2Cl_2 , δ): 3.61 (s, 3H), 3.98 (s, 3H), 8.60 (s, 1H). ¹³C NMR (125 MHz, CD_2Cl_2 , δ): 33.4, 41.2, 135.6, 141.7, 154.4, 156.8. IR (NaCl, thin film) (cm^{-1}): 2927, 1684, 1625, 1577. UV (EtOH) λ_{max} (log ϵ): 245 (3.8), 298(sh) (3.4), 552 (2.9). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_6\text{H}_8\text{N}_5\text{O}$, 166.0729; found, 166.0732.

Ethyl 6-(2-(*tert*-Butoxycarbonyl)hydrazinyl)-1,2,4-triazine-5-carboxylate (14).

In a 1 L round-bottom flask under an atmosphere of Ar, crude chlorotriazine **8** (11.5 g, from 14.5 g **7**) was dissolved in absolute EtOH (400 mL). *N*-(*tert*-Butoxycarbonyl)hydrazine⁹ (16.0 g, 121 mmol) was added and the solution was heated at reflux. After 2 h, the reaction was cooled to ambient temperature and the solvent was evaporated. The residue was partitioned between DCM (150 mL) and saturated NaHCO_3 (150 mL). The organic layer was then washed with saturated NaHCO_3 (75 mL). The aqueous layer (pH = 7) was basified to pH = 12 with 5 M aqueous NaOH (ca 10 mL). The now-basic layer was extracted with DCM (2 x 75 mL) and the organic extracts were combined, dried over MgSO_4 and evaporated. The residue was chromatographed using SiO_2 (6 x 20 cm) eluting with DCM followed by 1:9 (v/v) EtOAc:DCM to yield **14** as an orange, extremely viscous oil (11.5 g, 47% in 2 steps from **7**). ¹H NMR (300 MHz, CDCl_3 , δ): 1.47 (t, J = 7.2 Hz, 3H), 1.49 (bs, 9H), 4.52 (q, J = 7.2 Hz, 2H), 6.78 (bs, 1H), 9.05 (d, J = 3 Hz, 1H), 9.39 (s, 1H). ¹³C NMR (100 MHz, CDCl_3 , δ): 14.4, 28.4, 28.5, 63.7, 130.6, 150.7, 155.4, 156.0, 165.3. IR (NaCl, neat) (cm^{-1}): 3349, 3284, 2980, 2936, 1712, 1562, 1511. UV (EtOH) λ_{max} (log ϵ): 236 (3.9), 356 (3.4). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_4$, 284.1359; found, 284.1353.

1,2-Dihydro-3H-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (15).⁶

In a 500 mL round-bottom flask under an atmosphere of Ar, **14** (11.5 g, 40.6 mmol) was dissolved in DCM (300 mL). Thioanisole (6.5 mL, 55 mmol) was added followed by addition of trifluoroacetic acid (75 mL). The reaction was stirred for 5 h at ambient temperature and then evaporated to a constant weight. The residue was dissolved in MeOH (300 mL) and carefully neutralized with the aid of a pH meter¹⁰ to pH = 7.4 using crushed potassium hydroxide. After stirring for 2.5 h, silica (15 g) was added to the deep red solution and the solvent was removed. Chromatography using SiO_2 (4 x 10 cm) eluting with a solvent gradient (400 mL 1:1 EtOAc:hexanes; 400 mL EtOAc; 500 mL 1:9 MeOH:EtOAc; 1.5 L 2:1:1 MeOH:EtOAc:hexanes) yielded **15** as a bright red-orange solid (1.95 g, 35%). Triazinone **15** was conveniently manipulated as its HCl salt, which could be obtained by acidification of a methanolic solution of **15** with excess conc. HCl (aq), followed by evaporation to yield the bright yellow **15**•HCl (= **15a**).

15: Mp 140 °C dec. ¹H NMR¹¹ (400 MHz, CD_3OD , δ): 9.45 (s, 1H). ¹³C NMR (100 MHz, CD_3OD , δ): 127.4, 150.5, 151.5, 157.2. IR (NaCl, thin film) (cm^{-1}): 3485, 3365, 2922, 1643, 1510. UV (EtOH) λ_{max} (log ϵ): 238 (3.7), 252sh (3.6), 282sh (3.3), 377 (2.6), 499 (2.5). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_4\text{H}_4\text{N}_5\text{O}$, 138.0416; found, 138.0418.

Nostocine A (2) and Fluviol A (3).

In a flame-dried, 250 mL round-bottom flask under an atmosphere of Ar, **15**•HCl (0.337 g, 1.95 mmol) was dissolved in a 1:4 (v/v) mixture of MeOH: CH_3CN (50 mL). TMSCHN_2 (0.50 mL, 2.0 M, 1.0 mmol) was added in one portion and the reaction was stirred at ambient temperature for 30 min. The

⁸ Needles from DCM/hexanes.

⁹ Commercially available from Aldrich: *tert*-Butyl carbazate.

¹⁰ A 0.25 mL aliquot was removed from the reaction and was diluted with water (1 mL) prior to pH analysis.

¹¹ **15**•HCl: ¹H NMR (400 MHz, CD_3OD , δ): 9.75 (s, 1H); **15**[−]K⁺: ¹H NMR (400 MHz, CD_3OD , δ): 8.97.

solvent was then removed and CHCl_3 (100 mL) was added to the resulting residue. After sonication for 20 min, the suspension was filtered and the filter cake (which we believe is a mixture of **15** and **15**•HCl) was washed with additional CHCl_3 (50 mL) until the filtrate ran colorless. The yellow-red filter cake was evacuated to dryness (0.178 g) and dissolved in 1:4 (v/v) MeOH: CH_3CN (25 mL) under an atmosphere of Ar. TMSCHN_2 (0.30 mL, 2.0 M, 0.60 mmol) was added in one portion and the reaction was stirred at ambient temperature for 30 min. The solvent was then removed and CHCl_3 (25 mL) was added to the resulting residue. After sonication for 20 min, the suspension was allowed to settle and the supernatant was carefully removed by pipet. The procedure was repeated until the extract was colorless. The red residue was evacuated to dryness (0.105 g) and dissolved in 1:4 (v/v) MeOH: CH_3CN (15 mL) under an atmosphere of Ar. TMSCHN_2 (0.15 mL, 2.0 M, 0.30 mmol) was added in one portion and the reaction was stirred at ambient temperature for 30 min. The solvent was then removed and the solid was extracted with CHCl_3 as above. The filtrates and extracts from the three cycles were combined and evaporated yielding a purple-brown solid (0.300 g) consisting of multiple mono- and dimethylated products. The crude material was dissolved in MeOH (10 mL) and evaporated onto SiO_2 gel (1.5 g). SiO_2 gel chromatography (4 x 8 cm) eluting first with 1:1 (v/v) EtOAc:hexanes (500 mL) then 1:19 (v/v) MeOH:EtOAc (500 mL) yielded two major fractions; one yellow and one purple. The yellow fraction was further purified by MPLC using a Biotage 12+M SiO_2 column (8 g) eluting with EtOAc:hexanes (1:9→1:5, 120 mL linear gradient; 1:5→1:1, 120 mL linear gradient; 1:1 120 mL isocratic) to yield fluviol A (**3**) as a bright yellow solid (0.054 g, 18%). The purple fraction was further purified by MPLC using a Biotage 12+M SiO_2 column (8 g) eluting with MeOH:EtOAc (1:99→1:19, 240 mL linear gradient; 1:19, 120 mL isocratic) to yield nostocine A (**2**) as a deep purple solid (0.013 g, 4%).

Nostocine A (2): Mp¹² 168–170 °C (lit.¹³ 171–172 °C). ¹H NMR (400 MHz, CD_3OD , δ): 4.04 (s, 3H), 8.76 (s, 1H). ¹H NMR (400 MHz, CDCl_3 , δ): 4.03 (s, 3H), 8.66 (s, 1H). ¹³C NMR (125 MHz, CD_3OD , δ): 41.5, 138.6, 142.7, 154.6, 161.1. IR (NaCl, thin film) (cm^{-1}): 3300, 2964, 2930, 1677, 1622, 1577. UV (MeOH) λ_{max} (log ϵ): 240 (3.8), 290(sh) (3.4), 548(br) (3.0). UV (EtOH) λ_{max} (log ϵ): 240 (3.8), 283sh (3.4), 541 (br) (3.0). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_5\text{H}_6\text{N}_5\text{O}$, 152.0572; found, 152.0570.

Fluviol A (3): Mp 198–201 °C (lit.^{14a} fluviol A: 196–197 °C; lit.^{14b} normethylpseudoiodinine: 196 °C). ¹H NMR (400 MHz, CDCl_3 , δ): 4.24 (s, 3H), 9.77 (s, 1H), 10.2 (bs, 1H). ¹H NMR (400 MHz, CD_3OD , δ): 4.20 (s, 3H), 9.64 (s, 1H). ¹³C NMR (125 MHz, CDCl_3 , δ): 57.1, 126.0, 149.9, 152.1, 155.5. IR (NaCl, thin film) (cm^{-1}): 3134, 2978, 2908, 1586, 1558. UV (EtOH) λ_{max} (log ϵ): 228 (3.9), 283 (3.5), 372 (3.1). UV (CH_2Cl_2) λ_{max} (log ϵ): 223 (4.0), 278 (3.5), 282(3.5), 289sh (3.4), 362 (br) (3.2). HRMS-ESI (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_5\text{H}_6\text{N}_5\text{O}$, 152.0572; found, 152.0581.

Methylation of Fluviol A (3) to Give Pseudoiodinine Following the Procedure of Lindner and Schaden.¹⁵

In a 250 mL Erlenmeyer flask, fluviol A (**3**) (0.0750 g, 0.496 mmol) was stirred as a mostly dissolved suspension in Et_2O (10 mL). Freshly prepared diazomethane¹⁶ (40 mL, ~0.8 M, ~32 mmol) was added in one portion and the reaction was stirred for 2 h. Subsequent evaporation, followed by MPLC purification using a Biotage 25+M SiO_2 column (40 g) eluted with acetone:DCM (1:2, 510 mL isocratic; 1:1, 1020 mL isocratic) yielded a yellow-orange solid (**20**, 0.021 g, 25%),¹⁷ and a purple solid¹⁸ (0.0060 g,

¹² Small prisms from EtOAc.

¹³ Hirate, K.; Nakagami, H.; Takashina, J.; Mahmud, T.; Kobayashi, M.; In, Y.; Ishida, T.; Miyamoto, K. *Heterocycles* **1996**, *43*, 1513–1519. Spectral data reported for **Nostocine A**: ¹H NMR (270 MHz, CD_3OD , δ): 4.02 (s, 3H), 8.74 (s, 1H). ¹³C NMR (100 MHz, CD_3OD , δ): 42.3, 139.4, 143.5, 155.4, 161.8. IR (KBr) (cm^{-1}): 3482, 2463, 1676, 1627. UV (MeOH) λ_{max} (log ϵ): 239 (3.8), 277(sh) (3.5), 555 (3.0).

¹⁴ a) Smirnov, V. V.; Kiprianova, E. A.; Garagulya, A. D.; Esipov, S. E.; Dovjenko, S. A. *FEMS Microbiol. Lett.* **1997**, *153*, 357–361 and personal communication from Dovjenko, S. A. Spectral data reported for **fluviol A**: ¹H NMR (CDCl_3 , δ): 4.25 (s, 3H), 9.78 (s, 1H), 10.24 (s, 1H); ¹H NMR ($\text{DMSO}-d_6$, δ): 4.15 (s, 3H), 9.76 (s, 1H), 12.09 (s, 1H); ¹H NMR (acetone- d_6 , δ): 4.20 (s, 3H), 9.71 (s, 1H), 12.80 (s, 1H). UV (EtOH) λ_{max} (log ϵ): 233 (4.16), 280 (3.56), 283 (3.56), 375 (3.16). b) (ref. 15). Spectral data reported for **normethylpseudoiodinine**: IR (KBr) (cm^{-1}): 3100. UV (CH_2Cl_2) λ_{max} : 231, 277, 281, 287, 360, 402.

¹⁵ Lindner, H. J.; Schaden, G. *Chem. Ber.* **1972**, *105*, 1949–1955.

¹⁶ Prepared according to the procedure outlined in note 3 of Arndt, F. *Org. Synth.* **1943**, *Coll. Vol.* 2, 165–167.

¹⁷ This is the so-called hauptprodukt of Lindner and Schaden¹⁵, whose structure assignment was confirmed by methylation of **21**

7%) identical to pseudoiodinine. Crystallization of pseudoiodinine was accomplished by dissolving 0.0050 g in acetone (0.080 mL) and carefully layering hexanes (0.160 mL) on top. Deep black-purple crystals (0.003 g) slowly formed upon sitting at ambient temperature overnight.

20: Mp⁸ 154–155 °C (lit.¹⁷ 149 °C). ¹H NMR (400 MHz, CDCl₃, δ): 4.19 (s, 3H), 4.21 (s, 3H), 9.69 (s, 1H). ¹H NMR (400 MHz, CD₂Cl₂, δ): 4.15 (s, 3H), 4.18 (s, 3H), 9.62 (s, 1H). ¹³C NMR (100 MHz, CD₂Cl₂, δ): 34.6, 57.5, 125.4, 148.8, 151.5, 154.0. IR (NaCl, thin film) (cm⁻¹): 3067, 2982, 1574, 1552. UV (CH₂Cl₂) λ_{max} (log ε): 237 (4.0), 280 (3.5), 285(3.5), 295sh (3.4), 397 (br) (3.1). UV (EtOH) λ_{max} (log ε): 237 (4.1), 284 (3.5), 388 (3.2). HRMS-ESI (m/z): [M+H]⁺ calcd for C₆H₈N₅O, 166.0729; found, 166.0732.

Pseudoiodinine: Mp 120–123 °C (lit.¹⁵ 112 °C). ¹H NMR¹⁹ (500 MHz, CD₂Cl₂, δ): 4.27 (s, 3H), 4.41 (s, 3H), 8.95 (s, 1H). ¹³C NMR (125 MHz, CD₂Cl₂, δ): 43.0, 57.6, 139.0, 142.6, 146.0, 160.7. IR (NaCl, thin film) (cm⁻¹): 2988, 2924, 2851, 1586, 1537. UV (CH₂Cl₂) λ_{max} (log ε): 230 (3.6), 255 (3.5), 295 (3.2), 533 (2.6). UV (EtOH) λ_{max} (log ε): 220 (3.7), 254 (3.6), 295sh (3.4), 520 (2.6). HRMS-ESI (m/z): [M+H]⁺ calcd for C₆H₈N₅O, 166.0729; found, 166.0737.

1,2-Dihydro-1-methyl-3H-pyrazolo[4,3-e][1,2,4]triazin-3-one (21).⁶

In a 250 mL round-bottom flask under an atmosphere of Ar, crude chlorotriazine **8** (1.678 g) was dissolved in absolute EtOH (100 mL). Methylhydrazine (0.50 mL, 9.3 mmol) was added dropwise over 1.5 min and the solution was stirred at ambient temperature overnight. The resulting yellow solution was evaporated to yield a red-orange solid. The crude product was purified by chromatography using SiO₂ (4 x 10 cm) eluting first with 1:2 (v/v) EtOAc:hexanes (300 mL), followed by 1:9 (v/v) MeOH:EtOAc (500 mL) to yield **21** as a yellow-orange solid (0.634 g, 27% in 2 steps from **7**). Mp⁸ 199–200 °C. ¹H NMR (400 MHz, CD₃OD, δ): 4.09 (s, 3H), 9.61 (s, 1H). ¹H NMR (400 MHz, CDCl₃, δ): 4.18 (s, 3H), 9.66 (s, 1H). ¹³C NMR (125 MHz, CD₃OD, δ): 34.3, 127.0, 149.0, 151.6, 153.9. IR (NaCl, thin film) (cm⁻¹): 2997, 2924, 2848, 1574. UV (EtOH) λ_{max} (log ε): 237 (4.0), 283 (3.5), 405 (3.7). HRMS-ESI (m/z): [M+H]⁺ calcd for C₅H₆N₅O, 152.0572; found, 152.0568.

3-Methoxy-1-methyl-1H-pyrazolo[4,3-e][1,2,4]triazine (20).

In a flame-dried, 50 mL round-bottom flask under an atmosphere of Ar, **21** (0.0340 g, 0.225 mmol) was dissolved in a 9:1 (v/v) mixture of CH₃CN:MeOH (10 mL). The yellow solution was cooled in an ice-water bath and TMSCHN₂ (0.550 mL, 2.0 M, 1.1 mmol) was added dropwise over 1.5 min. After 20 min, the mixture was poured directly onto a plug of SiO₂ gel (2 x 4 cm). The plug was washed with 1:1 (v/v) MeOH:EtOAc (25 mL) and the eluate was evaporated to yield a yellow-orange solid. The crude product was purified by MPLC using a Biotage 12+M Si₂O column (8 g) eluting with EtOAc:hexanes (1:2→EtOAc, 240 mL linear gradient) to give a yellow solid (0.0223 g, 60%) identical to **20**, as well as an orange solid (0.0040 g, 10%) identical to **22**.

Methylation of Nostocine A (2) to Give Pseudoiodinine.

In a 50 mL Erlenmeyer flask, nostocine A (**2**) (0.0040 g, 0.026 mmol) was dissolved in Et₂O (3 mL). Freshly distilled diazomethane²⁰ (0.75 mL, ~0.08 M, ~0.05 mmol) was added and the reaction was stirred for 1 h. Subsequent evaporation, followed by MPLC purification using a Biotage 12+M SiO₂ column (8 g) eluting with MeOH:EtOAc (1:99, 120 mL isocratic; 1:99→1:19, 240 mL linear gradient;

(see below); Spectral data reported for the **hauptprodukt**: ¹H NMR (60 MHz, CDCl₃, δ): 4.22 (s, 3H), 4.26 (s, 3H), 9.72 (s, 1H). UV (CH₂Cl₂) λ_{max}: 234, 275, 280, 285, 291, 386, 410.

¹⁸ The material is red as a TLC spot and in dilute solution.

¹⁹ Ref. 15 reports the ¹H NMR spectrum (CH₂Cl₂) of **pseudoiodinine** as δ 4.38 (s, 3H), 4.58 (s, 3H), 9.26 (s, 1H). We believe the discrepancy between our chemical shifts and those of ref. 15 are due to an incorrectly calibrated permanent magnet in the original (ca 1972) work (the magnitude of the discrepancy is roughly proportional to the chemical shift). The UV/vis spectra and mps are in good agreement and the methods of preparation are identical. Other spectral data reported for **pseudoiodinine**: UV (CH₂Cl₂): 254, 295, 374, 536.

²⁰ a) de Boer, J.; Backer, H. J. *Org. Synth.* **1963**, *Coll. Vol. 4*, 250–253. b) Titrated as described in ref. 16.

1:19, 120 mL isocratic) yielded a purple solid (0.0014 g, 33%) identical to **23** and a red-purple solid (0.0004 g, 9%) identical to **19**.²¹

²¹ Fluvioi C^{14a} has been assigned structure **19**, but unlike synthetic **19** which is red (purple as a solid), fluvioi C is reported to be yellow and its reported UV/vis spectrum and melting point are at variance with those found for synthetic **19**. Consequently, we conclude that the structure assigned to fluvioi C is incorrect.

Based on its propensity to suffer demethylation to **3**, its reported violet color (**19** is purple as a solid), its mp (128 °C, dec; 120–123 °C for **19**) and its UV/vis spectrum {UV (EtOH) (log ϵ): 221 (4.08), 253 (3.94), 296 (3.59), 520 (3.06)}, we suspect fluvioi E^{14a} is identical to pseudoiodinine (**19**) and that the structure (**18**) assigned to fluvioi E is incorrect. Unfortunately, the ¹H NMR spectra of fluviois B–E have not been reported, so the identity of fluvioi E with pseudoiodinine cannot be proposed with complete certainty.

Selected Spectra

Ethyl 1,4,5,6-Tetrahydro-6-oxo-1,2,4-triazine-5-carboxylate (6).

Ethyl 1,6-Dihydro-6-oxo-1,2,4-triazine-5-carboxylate (7).

Ethyl 6-Chloro-1,2,4-triazine-5-carboxylate (8).

***N*-(*tert*-Butoxycarbonyl)-*N*-methylhydrazine (9).**

Ethyl 6-(2-(*tert*-Butoxycarbonyl)-2-methylhydrazinyl)-1,2,4-triazine-5-carboxylate (10).

1,2-Dihydro-2-methyl-3H-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (11).

3-Methoxy-2-methyl-2H-pyrazolo[4,3-*e*][1,2,4]triazine (12).

Ethyl 6-(2-(*tert*-Butoxycarbonyl)hydrazinyl)-1,2,4-triazine-5-carboxylate (14).

1,2-Dihydro-3H-Pyrazolo[4,3-*e*][1,2,4]triazin-3-one (15).

Nostocine A (2).

Figure 1. HMQC Data for 2.

Reproduction of the hmqc spectrum of **2** recorded on a Varian Unity INOVIA 500 MHz. The following key correlations are observed: $^1\text{H}(@4.04) \rightarrow ^{13}\text{C}(@41.5)$; $^1\text{H}(@8.76) \rightarrow ^{13}\text{C}(@142.7)$. This is consistent with the reported¹³ coupled carbon spectrum: ^{13}C NMR (100 MHz, CD_3OD , δ): 42.3 (q), 139.4 (s), 143.5 (d), 155.4 (s), 161.8 (s).

Figure 2. HMQC Data for 2 (Expansion).

Reproduction of the hmqc spectrum of **2** recorded on a Varian Unity INOVIA 500 MHz. The following key correlations are observed: $^1\text{H}(@4.04) \rightarrow ^{13}\text{C}(@41.5)$; $^1\text{H}(@8.76) \rightarrow ^{13}\text{C}(@142.7)$. This is consistent with the reported ^{13}C coupled carbon spectrum: ^{13}C NMR (100 MHz, CD_3OD , δ): 42.3 (q), 139.4 (s), 143.5 (d), 155.4 (s), 161.8 (s).

Figure 3. HMBC Data for 2.

Reproduction of the gHMBC spectrum of **2** recorded on a Varian Unity INOVIA 500 MHz. The following key correlations are observed: $^1\text{H}(@4.04) \rightarrow ^{13}\text{C}(@138.6)$; $^1\text{H}(@8.76) \rightarrow ^{13}\text{C}(@154.6)$. This is consistent with the reported ^{13}C HMBC spectrum: $^1\text{H}(@4.02) \rightarrow ^{13}\text{C}(@139.4)$; $^1\text{H}(@8.74) \rightarrow ^{13}\text{C}(@155.4)$.

Figure 4. HMBC Data for 2 (Expansion).

Reproduction of the gHMBC spectrum of **2** recorded on a Varian Unity INOVIA 500 MHz. The following key correlations are observed: $^1\text{H}(@4.04) \rightarrow ^{13}\text{C}(@138.6)$; $^1\text{H}(@8.76) \rightarrow ^{13}\text{C}(@154.6)$. This is consistent with the reported ^{13}C HMBC spectrum: $^1\text{H}(@4.02) \rightarrow ^{13}\text{C}(@139.4)$; $^1\text{H}(@8.74) \rightarrow ^{13}\text{C}(@155.4)$.

Fluviol A (3).

Pseudoiodinine (19).

Figure 5. HMQC Data for 19.

Reproduction of the hmqc spectrum of **19** recorded on a Varian Unity INOVIA 500 MHz spectrometer with temperature control set to -30 °C to minimize decomposition to fluviol A. The following key correlations are observed: $^1\text{H}(@4.27) \rightarrow ^{13}\text{C}(@57.6)$; $^1\text{H}(@4.41) \rightarrow ^{13}\text{C}(@43.0)$; $^1\text{H}(@8.95) \rightarrow ^{13}\text{C}(@142.6)$.

Figure 6. HMQC Data for **19 (Expansion).**

Reproduction of the hmqc spectrum of **19** recorded on a Varian Unity INOVIA 500 MHz spectrometer with temperature control set to $-30\text{ }^{\circ}\text{C}$ to minimize decomposition to fluviol A. The following key correlations are observed: $^1\text{H}(@4.27) \rightarrow ^{13}\text{C}(@57.6)$; $^1\text{H}(@4.41) \rightarrow ^{13}\text{C}(@43.0)$; $^1\text{H}(@8.95) \rightarrow ^{13}\text{C}(@142.6)$.

Figure 7. HMBC Data for 19.

Reproduction of the gHMBC spectrum of **19** recorded on a Varian Unity INOVIA 500 MHz spectrometer with temperature control set to -30 °C to minimize decomposition to fluvioi A. The following correlations are observed: $^1\text{H}(@4.27) \rightarrow ^{13}\text{C}(@160.7)$; $^1\text{H}(@4.41) \rightarrow ^{13}\text{C}(@139.0)$; $^1\text{H}(@8.95) \rightarrow ^{13}\text{C}(@146.0)$

Figure 8. HMBC Data for **19 (Expansion).**

Reproduction of the gHMBC spectrum of **19** recorded on a Varian Unity INOVIA 500 MHz spectrometer with temperature control set to $-30\text{ }^{\circ}\text{C}$ to minimize decomposition to fluviol A. The following correlations are observed: $^1\text{H}(\text{@}4.27) \rightarrow ^{13}\text{C}(\text{@}160.7)$; $^1\text{H}(\text{@}4.41) \rightarrow ^{13}\text{C}(\text{@}139.0)$; $^1\text{H}(\text{@}8.95) \rightarrow ^{13}\text{C}(\text{@}146.0)$

1,2-Dihydro-1-methyl-3H-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (21).

3-Methoxy-1-methyl-1H-pyrazolo[4,3-*e*][1,2,4]triazine (20).

1,2-Dihydro-1,2-dimethyl-3*H*-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (22).

2,7-Dihydro-2,7-dimethyl-3*H*-pyrazolo[4,3-*e*][1,2,4]triazin-3-one (23).