Synthesis S. Puvvala et al. Paper

First Total Synthesis of a Cytotoxic Derivative of the Natural Product Aaptamine

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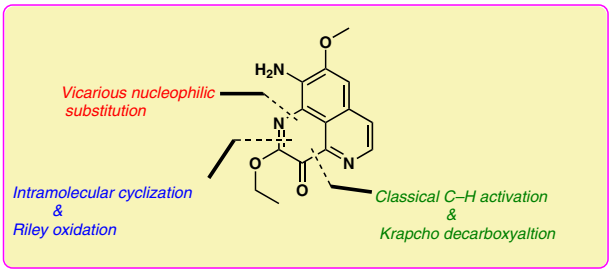

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Abstract A synthetic sequence to the benzonaphthyridinone framework is described. The key step is a one-pot, base-catalyzed vicarious nucleophilic substitution followed by ring closure. Additionally, the synthesis represents the application of a vicarious nucleophilic substitution in the total synthesis of a cytotoxic aaptamine derivative.

Key words total synthesis, heterocycles, vicarious aromatic substitution, cyclization, alkylation

Marine sponges have received a lot of attention from researchers, and are known to be a prolific source of novel chemicals with encouraging therapeutic potentials.[1] An ancestor aaptamine,[2] aaptamine I was isolated for the first time in 1981 by the Nakamura group from the marine sponge *Aaptos aaptos*.[3] Over the following years, aaptamine I and its congeners have been found in many other sponges (Class: *Desmospongiae*) from the Indian Ocean and the Red Sea, off the coasts of Australia, Brazil, Indonesia, and Malaysia, from the Caribbean Sea and from a South China Sea sponge. In particular, the genus *Aaptos* continues to be an abundant source of aaptamine alkaloids, which still spurs interest in finding new bioactive metabolites. Aaptamines and several members of the class (polycyclic quinones and hydroquinones) show a broad range of biological activities including antioxidant,[4a] enzymatic inhibition,[4b] antiviral,[4c] antimicrobial,[4d] antifungal,[4e] cytotoxic,[4f] and antidepressant.[4g] Among these, anticancer effects have been the most frequently reported for aaptamines.[4h–m]

The reported syntheses of aaptamine[5] use either the isoquinoline (AB) or quinoline (AC) components of the benzo[*de*][1,6]naphthyridine ring as a platform to build the third ring (Figure 1).

Among recently isolated aaptamines[6] **1**, **ii**, **iiia–d** and **iva–c**, compound **ii** possesses a piperidinyl group fused to a aaptamine moiety, whereas compounds **iiia–d** share an imidazole-fused 1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one skeleton (Figure 2).

To the best of our knowledge, there are no reports describing the synthesis of any of these compounds to date in the literature.[7] The presence of a novel isoquinoline skeleton and its potent cytotoxic activity encouraged us to look for an efficient synthetic pathway toward the synthesis of derivative **1**.

Looking for a rapid and versatile way to synthesize benzonaphthyridinones, we envisioned a retrosynthetic analysis following different disconnections as shown in (Scheme 1)

The crucial steps in our strategy are a vicarious nucleophilic substitution (VNS) followed by an intramolecular ester–amine coupling to construct the cyclic diketone intermediate **23**. The key substrate **20** for VNS cyclization can be obtained from chloroisoquinoline **14**, which in turn can be prepared from amide **7**. Amide **7** can be synthesized from functionalized 4-nitrobenzamide **5**. Thus, our first aim was to synthesize amide **5**, which can be easily prepared from commercially available 4-bromo-3-methylmethoxybenzene (**2**) (Scheme 2).

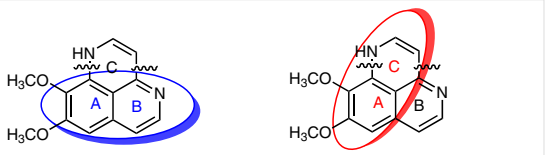


Figure 1 Reported syntheses to construct the AB and AC ring systems of aaptamine

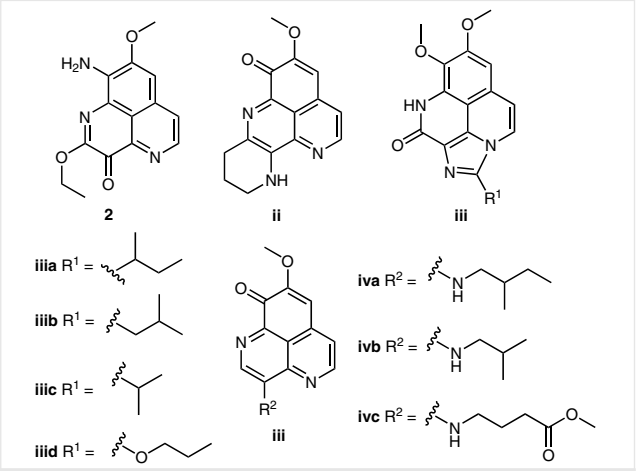
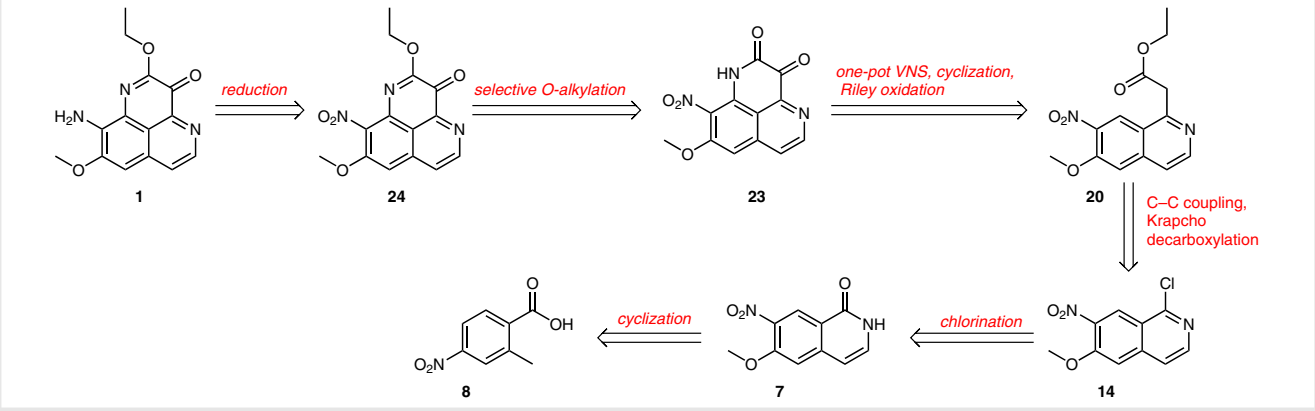


Figure 2 Structures of recently isolated aaptamine derivatives[6]

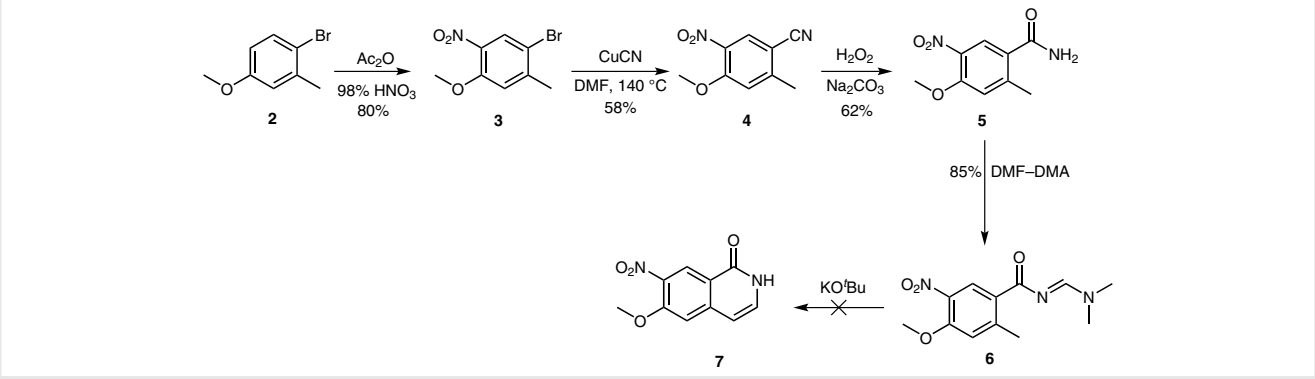
4-Bromo-3-methylmethoxybenzene (**2**) underwent nitration in the presence of concentrated HNO_3 and Ac_2O to give nitrobromobenzene **3** in quantitative yield. The copper-catalyzed S_NAr displacement of bromine with CuCN afforded cyanide **4**, which was subjected to oxidation using H_2O_2/Na_2CO_3 to give amide **5**. Treatment of **5** with dimethylformamide–dimethyl acetal (DMF–DMA) gave amidine **6** in good yield. However, the base-catalyzed cyclization of **6** to afford **7** was not encouraging due to the very low conversion and poor yields. Attempted optimization of the reaction by screening a number of strong bases, viz. NaOMe, NaH, LiHMDS and KO*t*-Bu proved unsuccessful.

Quickly, we changed our synthetic approach as shown in Scheme 3. The commercially available nitrobenzoic acid **8** on nitration gave dinitro compound **9**. The selective displacement of the *para*-nitro group with MeOH under basic conditions afforded compound **10**, which was further converted into a methyl ester using $SOCl_2$/MeOH. The obtained ester **11**[8] on treatment with 1-*tert*-butoxy-*N*,*N*,*N*′,*N*′-tetramethylmethanediamine afforded enamine **12**. The subsequent treatment of enamine **12** with electron-rich 2,4-dimethoxybenzylamine afforded the cyclized product **13**. The TFA-mediated debenzylation of intermediate **13** afforded the required intermediate **7**, which was further treated with phosphorous(V) oxychloride to give the key chloroisoquinoline **14** in good yield.[9,10]

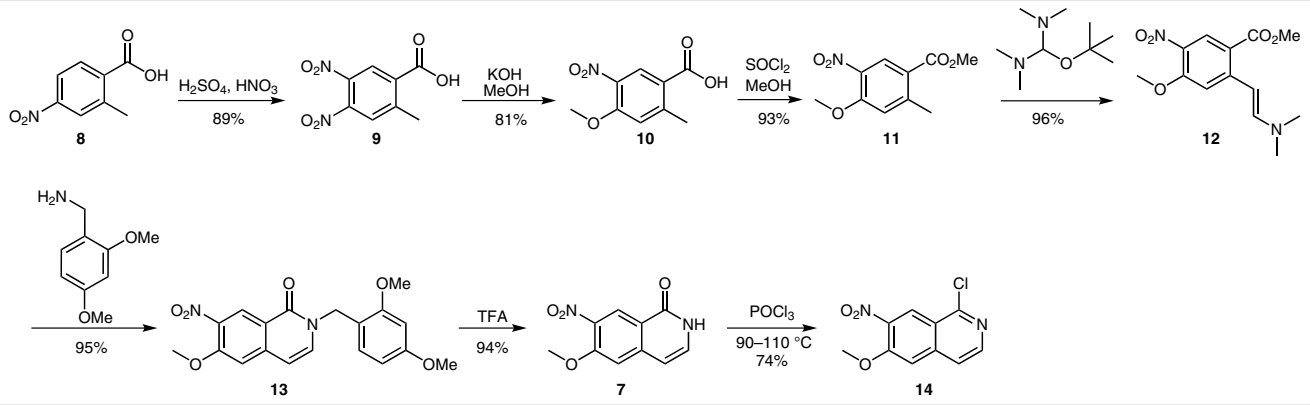
Initially, a straightforward synthesis of compound **18** was envisioned via the reaction sequence shown in path A (Scheme 4). The Suzuki coupling of **14** with methyl boronic



Scheme 1 Retrosynthetic analysis



Scheme 2 Attempted synthesis of precursor **7**

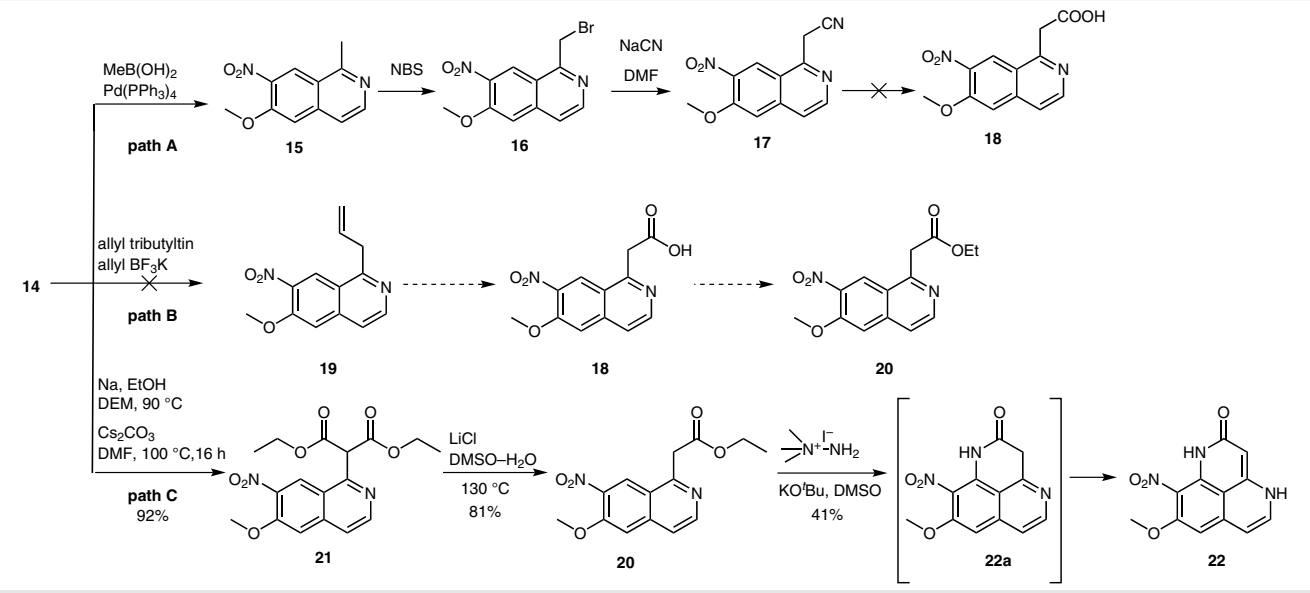


Scheme 3 Synthesis of precursor **14**

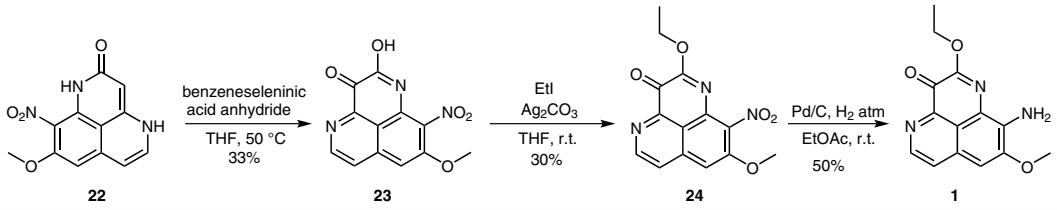
acid yielded compound **15**, which underwent allylic bromination and displacement of the resulting bromo intermediate **16** using sodium cyanide gave cyano intermediate **17**.[11,12] Surprisingly, hydrolysis of cyano intermediate **17** to give acid **18** was not effective under both acidic (HCl, THF, 50 °C) and basic (KOH, EtOH/H_2O) conditions. Alternatively, access to intermediate ester **20** via an initial Stille coupling starting from chloroisoquinoline **14** (path B, Scheme 4) to give compound **19** was quite low yielding, and further attempted oxidations ($NaIO_4$, $RuCl_3{\cdot}3H_2O$ in $MeCN/CCl_4/H_2O$ at r.t. to 60 °C) to give acid intermediate **18** were not promising. The synthetic challenge to access key intermediate **20** was finally overcome by employing a C–H activation route (path C, Scheme 4). For the classical condensation[13a] of diethyl malonate (DEM), different bases such as Na/EtOH, K_2CO_3 in DMF, KO^{t}Bu in THF, and NaH in DMSO were screened. Among these, Cs_2CO_3 in anhydrous DMF turned out to be best yielding compound **21** in excellent yield. Furthermore, Krapcho decarboxylation,[13b] using LiCl and DMSO at 130 °C afforded the key ester **20** in 81% yield. Ester **20** was observed as a mixture with its exocyclic isomer (an enamine), as was confirmed by variable temperature (VT) ^{1}H NMR spectroscopy.[14]

The key one-pot conversion into compound **22** was achieved successfully via a VNS reaction using trimethylhydrazinium iodide followed by base-catalyzed intramolecular cyclization. It was observed that this one-pot conversion led directly to compound **22** and not the expected intermediate **22a**, as was confirmed from HSQC and HMBC analysis.

Riley oxidation of intermediate **22** using SeO_2 in 1,4-dioxane[15a] at 90 °C for two hours gave compound **23** with low conversion. However, moderate yields were obtained by treatment with benzeneseleninic acid anhydride[15b] in dry



Scheme 4 Synthesis of 1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one (**22**)



Scheme 5 Synthesis of natural product **1**

THF (Scheme 5).[15c–e] The selective O-alkylation over N-alkylation of compound **23** was achieved by using Ag_2CO_3/ethyl iodide[16] to give intermediate **24** in 30% yield. Finally, reduction of the nitro group in intermediate **24** using Pd/C in EtOAc under hydrogen balloon pressure gave compound **1** in a reasonable 50% yield. ^{1}H NMR and ^{13}C NMR analyses were identical with reported isolated natural product data.[6]

In conclusion, a synthetic sequence to the benzonaphthyridinone skeleton has been developed, which could potentially open a synthetic pathway for the preparation of variously substituted compounds with a benzonaphthyridinone skeleton. The key steps involved in our strategy include a vicarious nucleophilic substitution followed by a ring closure. This approach is modular and rapid and may potentially be extended to synthesize biologically active natural product analogues or other useful building blocks.

All commercially available reagents used in this study were purchased from local suppliers, Sigma-Aldrich and Alfa Aesar and were used as such. Thin-layer chromatography was performed on pre-coated TLC silica gel 60 F254 aluminum plates (Merck). TLC plates were made visual under UV light (254 and 360 nm) or with iodine. Column chromatography was performed using Merck silica gel (230–400 mesh). Automated purifications were accomplished using Teledyne ISCO Combiflash companion and Grace reversal unless otherwise noted; all reactions were carried out under an atmosphere of argon (unless otherwise stated) in dried glassware using standard techniques. THF was distilled from Na/benzophenone. FA = formic acid. Analytical grade solvents were used without further drying/purification. Melting points were recorded using a Buchi melting point apparatus and are uncorrected. IR spectra (KBr pellets) were obtained using a Perkin Elmer Spectrum 2000 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz, Varian 400 MHz, and Varian 300 MHz spectrometers. Chemical shifts are reported in ppm with TMS as reference. Data are reported as follows: chemical shift, multiplicity, (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant(s), multiplicity. HRMS data was recorded using a Waters Q-Tof micro spectrometer under electrospray ionization mode.

2-Methyl-4,5-dinitrobenzoic Acid (9)

To concd H_2SO_4 (50 mL) at 0 °C was added concd HNO_3 (50 mL) dropwise and the mixture was allowed to stir at 0 °C for 10 min. Next, 2-methyl-4-nitrobenzoic acid (**8**) (9.0 g, 49.71 mmol) was added portionwise. The resulting reaction mixture was allowed to stir at r.t. for 16 h. The mixture was poured into ice-cold H_2O and the resulting solid was collected by filtration and dried to provide 2-methyl-4,5-dinitrobenzoic acid (**9**) (10 g, 89%) as an off-white solid.

IR (KBr): 3431, 3005, 1693, 1538, 1363, 1275, 1260, 764, 750 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 14.06 (br s, 1 H), 8.50 (s, 1 H), 8.22 (s, 1 H), 2.67 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.6, 147.6, 143.0, 138.7, 135.2, 128.0, 127.0, 20.9.

MS (ESI+): m/z = 225.10 [M – H]$^+$.

4-Methoxy-2-methyl-5-nitrobenzoic Acid (10)

2-Methyl-4,5-dinitrobenzoic acid (**9**) (5.0 g, 22.12 mmol) was added to KOH (6.206 g, 110.60 mmol) in MeOH (120 mL) and the resulting mixture was heated to 70 °C for 1.5 h. The reaction mixture was acidified with 2 M HCl and the solid was collected by filtration and dried to provide 4-methoxy-2-methyl-5-nitrobenzoic acid (**10**) (3.8 g, 81%) as a pale yellow solid; mp 249–251 °C.

IR (KBr): 3430, 3005, 1683, 1610, 1275, 1260, 764, 750 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.15 (br s, 1 H), 8.37 (s, 1 H), 7.32 (s, 1 H), 3.62 (s, 3 H), 2.64 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 166.3, 154.3, 148.1, 136.1, 127.9, 121.9, 117.0, 57.0, 21.9.

MS (ESI+): m/z = 212.16 [M + H]$^+$.

Methyl 4-Methoxy-2-methyl-5-nitrobenzoate (11)

To a solution of 4-methoxy-2-methyl-5-nitrobenzoic acid (**10**) (5.0 g, 23.69 mmol) in MeOH (50 mL) was added $SOCl_2$ (1.71 mL, 23.69 mmol) dropwise. The resulting mixture was heated to reflux for 5 h and then allowed to cool. The solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (60 mL) and sat. $NaHCO_3$ (60 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by chromatography on SiO_2 (15–25% EtOAc in hexanes) to provide methyl 4-methoxy-2-methyl-5-nitrobenzoate (**11**) (5.0 g, 93%) as a pale yellow solid; mp 130–133 °C.

IR (KBr): 3444, 2986, 2956, 1722, 1625, 1565, 1525, 1351, 1310, 1288, 1114, 986, 757 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.37 (s, 1 H), 7.35 (s, 1 H), 4.00 (s, 3 H), 3.83 (s, 3 H), 2.63 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.0, 154.5, 148.1, 136.1, 127.8, 120.6, 117.1, 57.0, 52.0, 21.6.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{10}H_{12}NO_5$: 226.0715; found: 226.0717.

Methyl (*E*)-2-[2-(Dimethylamino)vinyl]-4-methoxy-5-nitrobenzoate (12)

A mixture of methyl 4-methoxy-2-methyl-5-nitrobenzoate (**11**) (3.0 g, 13.32 mmol) and 1-*tert*-butoxy-*N*,*N*,*N*′,*N*′-tetramethylmethanediamine (3.3 mL, 15.99 mmol) was heated at 115 °C (no solvent) for 3.5 h. After cooling to r.t., the mixture was triturated with EtOAc/hexanes (1:6). The solid compound was collected by filtration and dried to provide of methyl (*E*)-2-[2-(dimethylamino)vinyl]-4-methoxy-5-nitrobenzoate (**12**) (3.6 g (96%) as an orange colored solid; mp 160–163 °C.

IR (KBr): 3444, 2950, 1708, 1630, 1590, 1544, 1315, 1260, 1109, 764, 749 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.40 (s, 1 H), 7.72 (d, *J* = 13.2 Hz, 1 H), 7.17 (s, 1 H), 6.28 (d, *J* = 13.2 Hz, 1 H), 3.98 (s, 3 H), 3.79 (s, 3 H), 2.98 (s, 6 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.7, 155.3, 150.0, 148.3, 131.1, 130.7, 113.8, 104.2, 92.4, 56.5, 51.7.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_{13}H_{17}N_2O_5$: 281.1137; found: 281.1156.

2-(2,4-Dimethoxybenzyl)-6-methoxy-7-nitroisoquinolin-1(2*H*)-one (13)

To a solution of methyl (*E*)-2-[2-(dimethylamino)vinyl]-4-methoxy-5-nitrobenzoate (**12**) (3.0 g, 10.71 mmol) in toluene (20 mL) was added 2,4-dimethoxybenzylamine (2.90 mL, 14.72 mmol). The resulting reaction mixture was stirred at 125 °C for 3.5 h during which the color changed from deep red to yellow. After cooling to r.t., the mixture was triturated with EtOAc/hexanes (1:2). The precipitate was collected by filtration to provide 2-(2,4-dimethoxybenzyl)-6-methoxy-7-nitroisoquinolin-1(2*H*)-one (**13**) (3.8 g, 95%) as a yellow solid; mp 225–228 °C.

IR (KBr): 3445, 2956, 2848, 1722, 1626, 1604, 1507, 1261, 1275, 764, 750 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.60 (s, 1 H), 7.57 (d, *J* = 7.3 Hz, 1 H), 7.49 (s, 1 H), 7.05 (d, *J* = 8.3 Hz, 1 H), 6.64 (d, *J* = 7.3 Hz, 1 H), 6.59 (d, *J* = 2.0 Hz, 1 H), 6.48 (dd, *J* = 8.3, 2.4 Hz, 1 H), 5.01 (s, 2 H), 4.01 (s, 3 H), 3.81 (s, 3 H), 3.74 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 160.3, 159.9, 158.0, 154.3, 141.9, 138.3, 137.0, 130.2, 125.2, 117.9, 116.5, 109.1, 104.6, 103.7, 98.4, 57.0, 55.5, 55.2, 46.6.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_{19}H_{19}N_2O_6$: 371.1243; found: 371.1233.

6-Methoxy-7-nitroisoquinolin-1(2*H*)-one (7)

A solution of 2-(2,4-dimethoxybenzyl)-6-methoxy-7-nitroisoquinolin-1(2*H*)-one (**13**) (2.5 g, 6.75 mmol) in TFA (40 mL) was stirred at 85 °C for 2.5 h. The reaction mixture was cooled to r.t. and the TFA was removed under reduced pressure. The crude product was rinsed once with MeOH (40 mL) and dried under high vacuum. The resulting solid was triturated with EtOAc (30 mL) and collected by filtration to provide 6-methoxy-7-nitroisoquinolin-1(2*H*)-one (**7**) (1.4 g, 94%) as a yellow solid; mp 305–308 °C.

IR (KBr): 3304, 3170, 3050, 2932, 1821, 1655, 1637, 1564, 1518, 1359, 1309, 1295, 1046, 869, 688 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 11.47 (br s, 1 H), 8.57 (s, 1 H), 7.50 (s, 1 H), 7.36 (t, *J* = 6.4 Hz, 1 H), 6.58 (d, *J* = 6.8 Hz, 1 H), 4.02 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 160.8, 154.4, 143.0, 138.1, 133.1, 124.9, 118.5, 109.2, 103.7, 57.0.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_{10}H_9N_2O_4$: 221.0562; found: 221.0567.

1-Chloro-6-methoxy-7-nitroisoquinoline (14)

A solution of 6-methoxy-7-nitroisoquinolin-1(2*H*)-one (**7**) (3.0 g, 13.63 mmol) in $POCl_3$ (40 mL) was stirred at 90–100 °C for 3 h. The reaction mixture was cooled to r.t. and the excess $POCl_3$ removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (2 × 30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (30–50% EtOAc in hexanes) to provide 1-chloro-6-methoxy-7-nitroisoquinoline (**14**) (2.4 g, 74%) as a yellow solid; mp 198–201 °C.

IR (KBr): 3429, 2927, 1629, 1521, 1377, 1350, 1303, 1204, 1084, 955, 864, 722 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.75 (s, 1 H), 8.39 (d, *J* = 5.9 Hz, 1 H), 7.85–7.92 (m, 2 H), 4.07 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 152.3, 150.8, 144.3, 141.7, 140.1, 122.9, 120.2, 119.6, 108.9, 57.3.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_{10}H_8N_2O_3Cl$: 239.0223; found: 239.0235.

Diethyl 2-(6-Methoxy-7-nitroisoquinolin-1-yl)malonate (21)

A mixture of 1-chloro-6-methoxy-7-nitroisoquinoline (**14**) (2.0 g, 8.40 mmol), diethyl malonate (DEM) (2.54 mL, 16.80 mmol) and Cs_2CO_3 (5.45 g, 16.80 mmol) in DMF (20 mL) was stirred at 100 °C for 6 h. After cooling to r.t., the reaction mixture was filtered, diluted with H_2O (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (20–50% EtOAc in hexanes) to provide diethyl 2-(6-methoxy-7-nitroisoquinolin-1-yl)malonate (**21**) (2.8 g, 92%) as an off-white solid; mp 98–101 °C.

IR (KBr): 3448, 2984, 2940, 1732, 1633, 1532, 1372, 1277, 1259, 1202, 1045, 866, 764, 750 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.68 (s, 1 H), 8.53 (d, *J* = 5.9 Hz, 1 H), 7.84 (d, *J* = 5.5 Hz, 1 H), 7.81 (s, 1 H), 6.07 (s, 1 H), 4.20 (q, *J* = 7.1 Hz, 4 H), 4.06 (s, 3 H), 1.18 (t, *J* = 7.0 Hz, 6 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.9, 154.6, 151.3, 144.2, 141.3, 138.8, 122.2, 120.3, 120.0, 108.7, 61.4, 57.0, 13.8.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_{17}H_{19}N_2O_7$: 363.1192; found: 363.2824.

Ethyl 2-(6-Methoxy-7-nitroisoquinolin-1-yl)acetate (20)

A solution of diethyl 2-(6-methoxy-7-nitroisoquinolin-1-yl)malonate (**21**) (2.0 g, 5.52 mmol) in DMSO (20 mL), LiCl (0.245 g, 5.85 mmol) and H_2O (0.17 mL, 9.83 mmol) was stirred at 130 °C for 6 h. After cooling to r.t., the mixture was diluted with H_2O (40 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 (20–30% EtOAc in hexanes) to provide ethyl 2-(6-methoxy-7-nitroisoquinolin-1-yl)acetate (**20**) (1.3 g, 81%) as orange solid; mp 170–173 °C.

IR (KBr): 3445, 2989, 1629, 1609, 1275, 1260, 764, 750 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ (mixture with the exocyclic isomer) = 12.11 (d, J = 3.9 Hz, 1 H), 8.79 (s, 1 H), 8.58 (s, 1 H), 8.48 (d, J = 5.9 Hz, 1 H), 7.70–7.83 (m, 2 H), 7.46 (t, J = 6.4 Hz, 1 H), 6.42 (d, J = 6.8 Hz, 1 H), 5.48 (s, 1 H), 4.38 (s, 2 H), 4.10 (qd, J = 7.1, 1.2 Hz, 6 H), 3.98 (s, 3 H), 1.05–1.28 (m, 6 H).

^{1}H NMR at 90 °C

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.70 (s, 1 H), 8.46 (d, J = 5.9 Hz, 1 H), 7.73 (d, J = 5.9 Hz, 1 H), 7.70 (s, 1 H), 4.34 (s, 2 H), 4.11 (q, J = 6.8 Hz, 2 H), 4.05 (s, 3 H), 1.16 (t, J = 7.1 Hz, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ (mixture with the exocyclic isomer) = 169.8, 169.5, 156.1, 153.3, 151.2, 150.1, 144.5, 141.1, 139.7, 138.6, 138.5, 132.6, 123.2, 121.9, 120.6, 119.2, 116.5, 109.2, 108.3, 104.1, 75.6, 60.5, 58.1, 56.9, 56.8, 41.0, 14.5, 13.9.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{14}H_{15}N_2O_5$: 291.0981; found: 291.0989.

8-Methoxy-9-nitro-1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one (22)

To a solution of ethyl 2-(6-methoxy-7-nitroisoquinolin-1-yl)acetate (**20**) (1.5 g, 5.17 mmol) in dry DMSO (30 mL) was added trimethylhydrazinium iodide (TMHI) (1.25 g, 6.20 mmol) and the mixture was stirred for 5 min. KO*t*-Bu (1.45 g, 12.92 mmol) was added portionwise and the resulting mixture was poured onto an ice-cold solution of 50% AcOH and extracted with 10% MeOH in EtOAc (2 × 100 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on C-18 (30–50% MeCN/0.1 M FA) to provide 8-methoxy-9-nitro-1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one (**22**) (0.55 g, 41%) as a yellow solid; mp 302–306 °C.

IR (KBr): 3446, 3005, 1627, 1275, 1260, 764, 750 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.39 (br s, 1 H), 8.44 (br s, 1 H), 7.29 (d, J = 6.0 Hz, 1 H), 6.61 (s, 1 H), 6.19 (s, 1 H), 5.34 (s, 1 H), 3.91 (s, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 161.6, 156.3, 148.4, 140.4, 136.6, 135.6, 121.6, 107.4, 103.9, 97.1, 87.0, 56.8.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{12}H_{10}N_3O_4$: 260.0671; found: 260.0680.

2-Hydroxy-8-methoxy-9-nitro-3*H*-benzo[*de*][1,6]naphthyridin-3-one (23)

To a stirred solution of benzeneseleninic acid anhydride (2.77 g, 7.72 mmol) in dry THF (40 mL) at 50 °C was slowly added a solution of 8-methoxy-9-nitro-1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one (**22**) (1.0 g, 3.86 mmol) in THF (10 mL) dropwise. The resulting mixture was stirred at the same temperature for 1 h (until the starting material had been consumed). The mixture was cooled to r.t., diluted with EtOAc (100 mL) and washed with H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by reverse-phase chromatography on C-18 using a Grace reversal instrument (40–50% MeCN/0.01 M FA) to provide 8-methoxy-9-nitro-3*H*-benzo[*de*][1,6]naphthyridine-2,3-dione (**23**) (0.350 g, 33%) as an orange solid; mp 241–273 °C.

IR (KBr): 3225, 3087, 2923, 1703, 1629, 1541, 1446, 1378, 1346, 1310, 1086, 874 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.91 (br s, 1 H), 8.85 (d, J = 5.3 Hz, 1 H), 8.06 (d, J = 5.5 Hz, 1 H), 7.36 (s, 1 H), 4.02 (s, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 176.2, 157.5, 152.2, 147.7, 146.8, 137.1, 128.8, 128.1, 124.1, 115.1, 99.2, 57.1.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{12}H_8N_3O_5$: 274.0464; found: 274.0466.

2-Ethoxy-8-methoxy-9-nitro-3*H*-benzo[*de*][1,6]naphthyridin-3-one (24)

To a stirred solution of 8-methoxy-9-nitro-3*H*-benzo[*de*][1,6]naphthyridine-2,3-dione (**23**) (0.25 g, 0.91 mmol) in THF (10 mL) was added silver carbonate (0.75 g, 2.74 mmol) and ethyl iodide (0.22 mL, 2.74 mmol) at r.t. The resulting mixture was stirred at r.t. for 6 h and then diluted with H_2O (50 mL) and extracted with EtOAc (2 × 25 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (100–200 mesh, 40–60% EtOAc in hexanes) to afford compound **24** (0.083 g, 30%) as a pale yellow solid; mp 134–136 °C.

IR (KBr): 3446, 2981, 2932, 1851, 1738, 1698, 1620, 1602, 1541, 1464, 1385, 1284, 1192, 1087, 869 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.06 (d, J = 5.3 Hz, 1 H), 7.93 (d, J = 5.3 Hz, 1 H), 7.18 (s, 1 H), 4.59 (q, J = 7.0 Hz, 2 H), 4.09 (s, 3 H), 1.50 (t, J = 7.2 Hz, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.5, 158.6, 153.3, 148.5, 145.8, 138.9, 136.8, 131.3, 124.8, 117.6, 103.8, 65.2, 57.0, 13.8.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{14}H_{12}N_3O_5$: 302.0777; found: 302.0787.

9-Amino-2-ethoxy-8-methoxy-3*H*-benzo[*de*][1,6]naphthyridin-3-one (1)

To stirred solution of 2-ethoxy-8-methoxy-9-nitro-3*H*-benzo[*de*][1,6]naphthyridin-3-one (**24**) (0.02 g, 0.0062 mmol) in EtOAc (5 mL) was added 10% Pd/C (5% w/w). The reaction mixture was stirred under a H_2 atm (20 psi) for 6 h (until the starting material had been consumed). The reaction mixture was filtered through a pad of Celite, the filtrate was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by reverse-phase chromatography on C-18 using a Grace reversal instrument (20–30% MeCN/0.01 M FA) to afford 9-amino-2-ethoxy-8-methoxy-3*H*-benzo[*de*][1,6]naphthyridin-3-one (**1**) (9 mg, 50%) as a red solid; mp 150–153 °C.

IR (KBr): 3383, 2924, 2853, 2347, 1600, 1482, 1362, 1266, 1096, 789 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.73 (d, J = 4.5 Hz, 1 H), 7.93 (d, J = 4.5 Hz, 1 H), 7.31 (s, 1 H), 7.26 (br s, 2 H), 4.51 (q, J = 7.0 Hz, 2 H), 4.04 (s, 3 H), 1.39 (t, J = 7.0 Hz, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.0, 155.1, 152.3, 144.5, 141.8, 141.7, 132.0, 124.2, 118.2, 113.8, 103.5, 62.0, 56.4, 14.2.

HRMS (ESI+): m/z [M + H]$^+$ calcd for $C_{14}H_{14}N_3O_3$: 272.1035; found: 272.1042.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0036-1588752.

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