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Toward the Total Synthesis of Alpkinidine: Michael Addition to Isoquinolinetrione CE Ring-System Synthons

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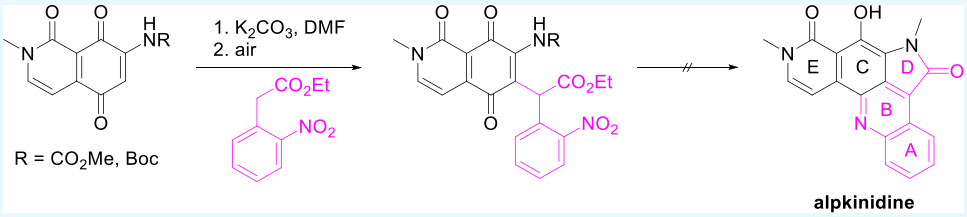

ABSTRACT: Strategies toward the total synthesis of the marine pyrroloacridine alkaloid alpkinidine have been explored, focusing on linking quinonoid CE ring-system synthons with the A ring, followed by condensation to form the B and D rings. The key Michael addition of the ester enolate derived from ethyl *o*-nitrophenylacetate to 2-methylisoquinoline-1,5,8(2*H*)-trione proceeded with the wrong regiochemistry. This issue was addressed by incorporating the D-ring nitrogen at an earlier stage, affording advanced intermediates possessing the complete carbon skeleton of alpkinidine. However, attempts to close the D and B rings were unsuccessful. The novel isoquinolinetriones reported here, and the general strategy of connecting CE- and A-ring synthons through Michael additions, may be useful in the synthesis of other pyrrolo- and pyridoacridines, in particular the anticancer lead neoamphimedine and analogues.

INTRODUCTION

In the preceding paper in this issue, we reported concise syntheses of 6-halo and 6,7-dichloroisoquinolinetriones matching the CE ring system of the marine secondary metabolite alpkinidine (**10**) and unsuccessful efforts to elaborate these to the natural product (Scheme 1). We previously described an efficient route to the model pentacyclic pyrroloacridine **5** by Michael substitution of 2,3-dichloronaphthoquinone (**1**) with the anion derived from ethyl *o*-nitrophenylacetate (**2**) to give **3**, D-ring formation through nucleophilic lactamization with methylamine, and reductive cyclization of **4** to close the B ring.[1] Despite this precedent, attempts to effect an analogous Michael substitution of dichloroisoquinolinetrione **6** were unsuccessful.[2] Formation of the key carbon−carbon bond ultimately comprising the BD-ring fusion was achieved in the analogous reaction of monobromide **8**; however, we were not able to progress adduct **9** toward alpkinidine (**10**).

Herein, we report a continuation of our efforts toward the total synthesis of alpkinidine, specifically the use Michael addition (as opposed to substitution) to form the key carbon−carbon bond highlighted in red in Scheme 1.

RESULTS AND DISCUSSION

At the outset, attempts to use Michael addition reactions to couple 1,4-naphthoquinone (**11**) and *o*-nitrophenylacetonitrile (**12**) had been unsuccessful, producing what appeared to be oligomeric products instead of the expected hydroquinone **13** or quinone **14** adducts (Scheme 2),[1] hence our prior focus on Michael substitution of haloquinones **6** and **8** (Scheme 1).[2]

In an effort to resurrect the general strategy of connecting the CE- and A-ring systems of potential precursors to alpkinidine, through the reaction of carbanions with quinone electrophiles,[2] the reaction of ethyl *o*-nitrophenylacetate (**2**) with isoquinolinetrione **15**[2] was investigated and indeed provided a hydroquinone adduct in modest yield (Scheme 3). Unfortunately, heteronuclear multiple bond correlation (HMBC) spectroscopy revealed the product to be the undesired regioisomer **16**. In an attempt to elaborate **16** through to the isomer of alpkinidine with an inverted E-ring **19**, the hydroquinone was oxidized, and quinone **17** was treated with methylamine in air, but the expected lactam **18** was not detected in the complex mixture of products formed.

The successful Michael addition to give **16** was encouraging and inspired efforts to achieve the opposite regioselectivity required for the synthesis of alpkinidine (**10**). Isoquinoline-

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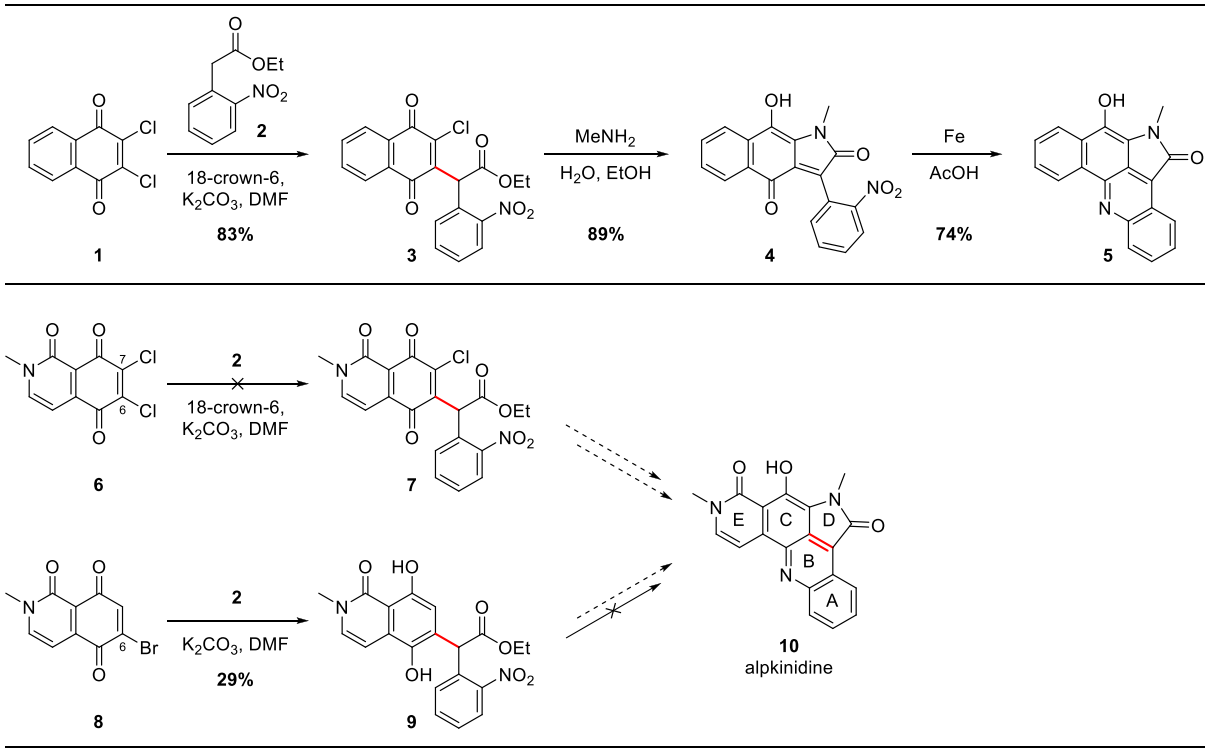
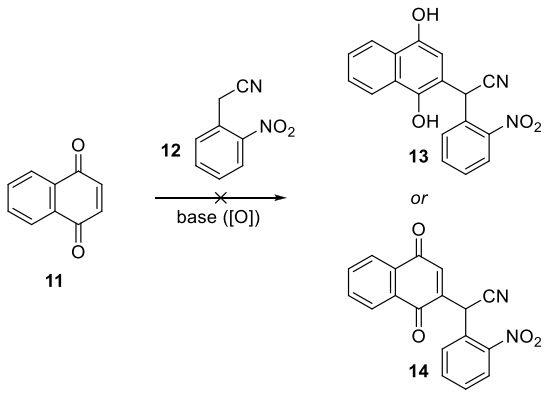
Scheme 1. Summary of Our Previous Work toward Alpkinidine[a]



[a]Please see the preceding paper for more background information on alpkinidine[3] and related synthetic work by others.[4−6]

Scheme 2. Unsuccessful Attempted Model Michael Addition[1]



1,5,8-(2*H*)-trione (**30**) had not been reported, but its hydroxypyridine tautomer **31** was assumed to be more stable by virtue of a strong intramolecular hydrogen bond (Scheme 4). It was hypothesized that this hydrogen bond might promote the desired regiochemistry by withdrawing electron density from the *peri*-carbonyl, thereby increasing electrophilicity at C6, and potentially provide anchimeric assistance for the Michael addition reaction, that is, through general acid catalysis under equilibrium deprotonation conditions. N-Methylation of the E-ring nitrogen could then follow at a later stage en route to alpkinidine. Hence, we set out to prepare **31**.

Amidation of 2,5-diacetoxybenzoic acid (**20**)[7] with commercial aminoacetal **21** gave **22**, along with the partially deacetylated product **23** (Scheme 4). The mixture was treated with concentrated H_2SO_4 but, while the analogous *N*-methylamide cyclized under these conditions,[2] in this case the isoquinolone **26** could not be detected in the complex mixture of products formed. In an attempt to avoid phenol protecting groups, methyl 2,5-dihydroxybenzoate (**24**) was heated with neat amino acetal **21**, providing the amide **25** in acceptable yield. Annulation in neat sulfuric acid was successful in this instance but provided the hydroquinone **26** in poor yield. The Pomeranz−Fritsch-like cyclization was more efficient with the dimethyl ether **28**, derived from 2,5-dimethoxybenzoic acid (**27**), and smooth Prey demethylation[8] of **29** was achieved. However, attempts to oxidize the hydroquinone **26** to the quinone **30**/**31** gave complex mixtures of products, and thus, this line of investigation was abandoned.

The regiochemical issues described above (and in Scheme 3) could potentially be solved by incorporation of the 7-aminomethyl group at an earlier stage of the synthesis (Scheme 5). Michael addition of ethyl *o*-nitrophenylacetate (**2**) to a suitably protected aminoquinone **32** would give hydroquinone **33**, which upon deprotection of the amino group, would be expected to cyclize to give lactam **34**. Reduction of the nitro group followed by aerial oxidation would then allow cyclodehydration to give alpkinidine (**10**).

Serendipity played a role in allowing us to explore the general strategy set out in Scheme 5. During an attempt to oxidatively demethylate **35**,[2] Ag_2O was mistakenly used instead of AgO (Scheme 6). Unsurprisingly, no quinone **15** was isolated from this reaction, but the electron-rich ring system was sufficiently reactive to undergo nitration with the nitric acid. Two regioisomers were isolated, with X-ray crystallography confirming the identities as the 7-nitroisoquinolone **36** and 4-nitroquinoline **37** isoquinolones. A similar yield of the major regioisomer **36** was obtained when the reaction was repeated in the absence of the silver(I) oxide.

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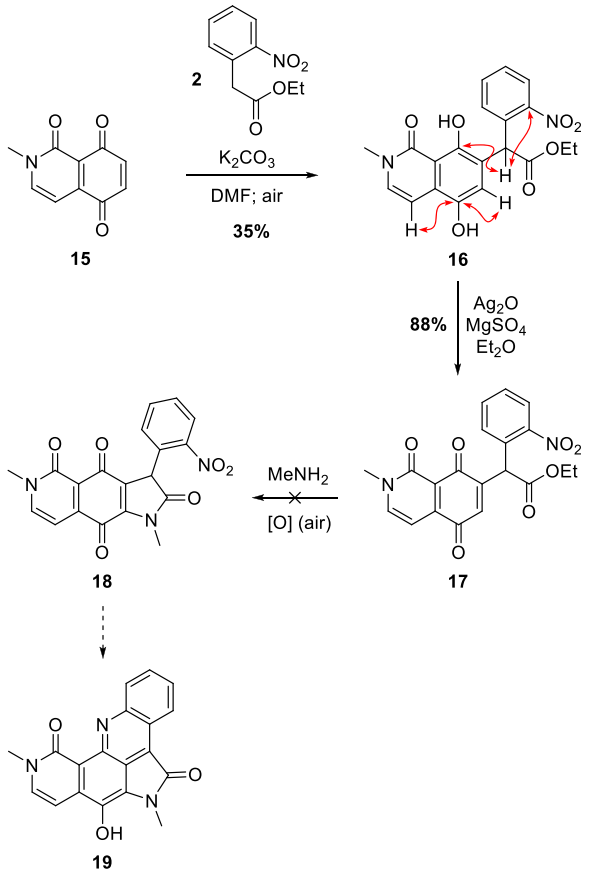
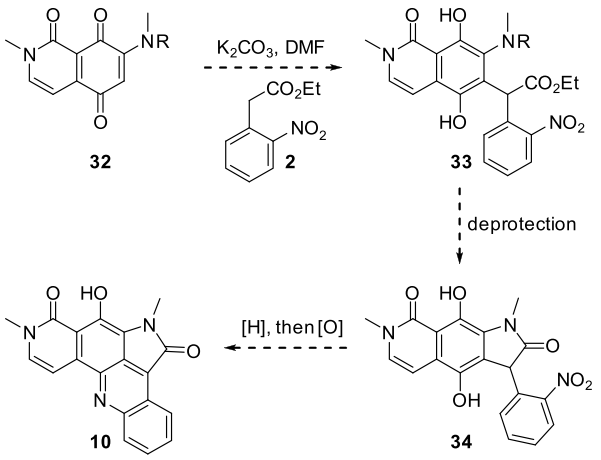
Scheme 3. Michael Addition of 2 to Quinone 15 and Attempted Elaboration toward Alpkinidine Isomer 19[a]



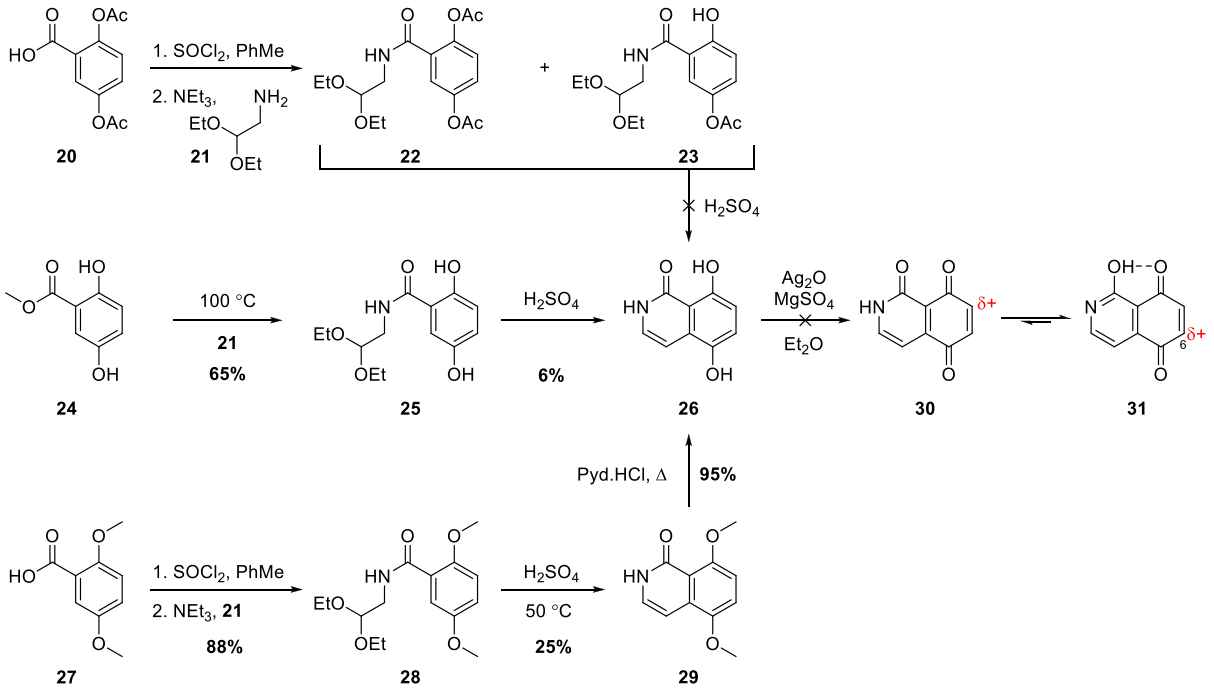
[a]The red double-headed arrows indicate HMBC correlations, which established the regiochemistry of the Michael addition.

Scheme 5. Proposed Regioselective Synthesis of Alpkinidine



Surprisingly, when this reaction was repeated several years later, the ratio of **36**:**37** was inverted (see yields in brackets), as confirmed by comparison of the ^{1}H NMR spectra of the crude product from each reaction. The only possible explanation we could posit for the change in product ratio is differing water content of the nitric acid/acetone. The variable outcomes for this reaction led us to devise alternative routes to 7-nitrogenated isoquinolones; the first is set out in Scheme 7. Formylation of *p*-methoxyphenol (**38**),[9] or *peri* demethylation of 2,5-dimethoxybenzaldehyde[10] (see the Experimental Section), gave salicylaldehyde **39**, which was regioselectively nitrated providing **40**.[11] O-Methylation was followed by oxidation of **41** to the benzoic acid **42**, which was converted to the acid chloride and amidated with secondary amine **43**.[2] Unsurprisingly, cyclization of benzamide **44** in sulfuric acid was not as facile as for the des-nitro analogue,[2] and a temperature of 100 °C was required to achieve an acceptable yield of the isoquinolone **36**.

Scheme 4. Attempted Synthesis of 1-Hydroxyisoquinoline-5,8-dione (31)



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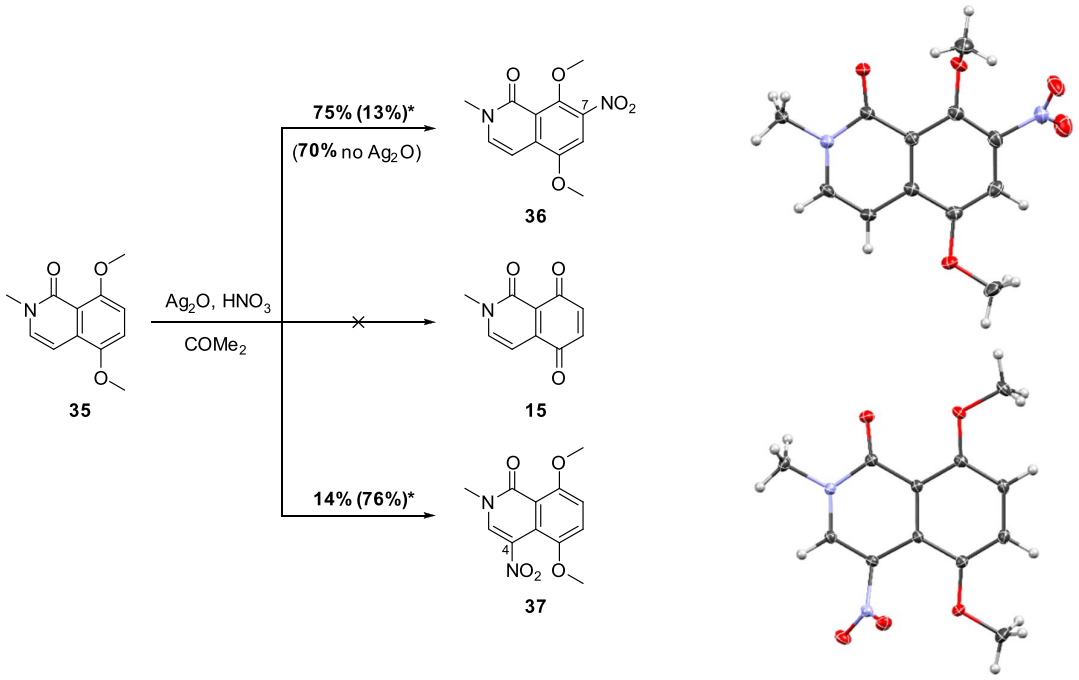
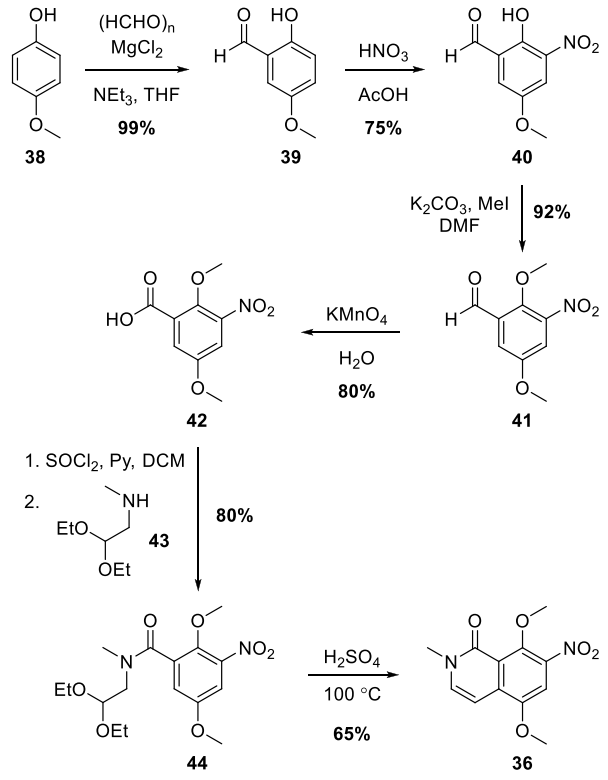
Scheme 6. Serendipitous Regioselective Nitration of 35[a]



[a]Astersisks (*): Yields in brackets are from the same reaction conducted several years later.

Scheme 7. Alternative Synthesis of 7-Nitroisoquinolone 36



Smooth reduction of **36** to the aniline **45** was effected with Fe or $SnCl_2$, and followed by derivatization, affording the acetanilide **46** or carbamates **47** and **48** (Scheme 8). The carbamates were also N-methylated providing **49** and **50**. It became apparent that these compounds could be accessed more efficiently from **35**[2] (Scheme 8). Thus, selective demethylation *peri* to the carbonyl provided phenol **51**, which underwent regioselective nitration affording **52**, as confirmed by 2D NMR experiments. Reduction to the aniline **53** was followed by conversion to methyl carbamate **54**; the use of pyridine as solvent/base avoided O-carboxymethylation, which was a competing side reaction with the stronger base triethylamine. Finally, N,O-dimethylation afforded **49**.

An attempted oxidative demethylation of **45** (Scheme 9) gave an intractable mixture, in line with previous observations from reactions of dimethoxyanilines with CAN.[12] In any case, it was expected that the amino group in **55** would be so electron-donating as to preclude a subsequent Michael addition at C6, hence the protection with electron-withdrawing groups. The oxidative demethylation of the secondary acetamide **46** and carbamates **47** and **48** proceeded as expected to the give corresponding quinones **56**−**58**, respectively. In surprising contrast, the analogous reactions of the tertiary carbamates **49** and **50** failed to give the expected quinones **59** and **60**. In the case of the methyl carbamate **49**, dinitration was instead observed, affording **61** in moderate yield. Related nitrations of electron-rich aromatic compounds with CAN have been reported.[13−16] No reaction of **49** was observed with phenyliodine(III)-bis(trifluoroacetate) (PIFA).[17] When the *t*-butyl carbamate **50** was treated with CAN, cleavage of the Boc group to give aniline **62** was the only reaction observed. CAN-mediated cleavage of Boc groups is well known;[18] however, a sensible explanation for the difference in reactivity between the secondary (**47**/**48**) and tertiary (**49**/**50**) carbamates escapes us.

With appropriately nitrogenated quinones in hand, the key Michael addition step was investigated (Scheme 10). The reaction of **2** with acetamide **56** gave a complex mixture of products, but encouragingly, Michael additions to the methyl (**57**) and *t*-butyl (**58**) carbamates were successful; the initially formed hydroquinone adducts presumably oxidized during workup to the isolated quinones **64** and **65**, respectively. A single attempt to deprotect **64** failed to give the expected

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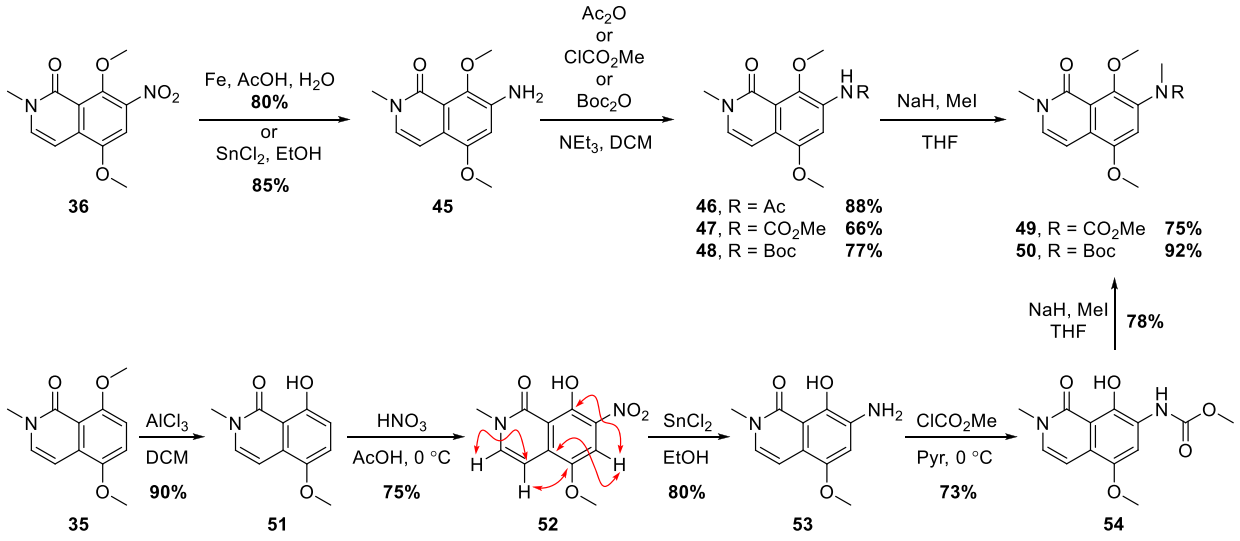
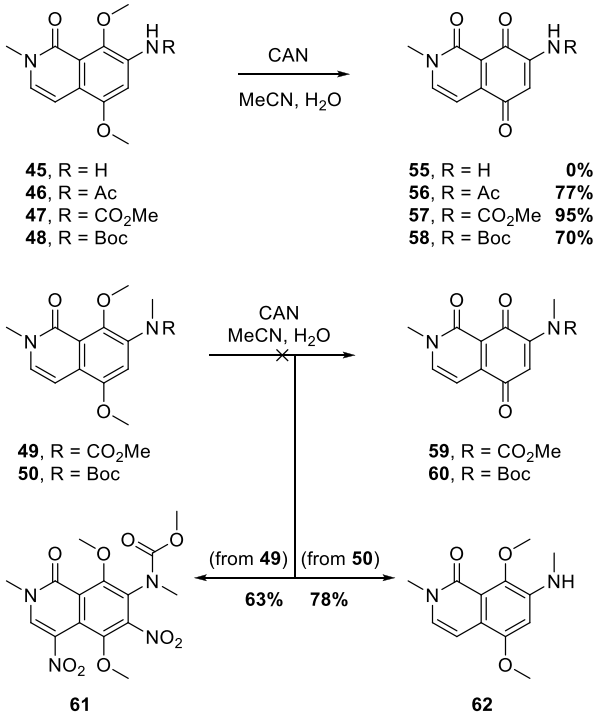
Scheme 8. Preparation of Protected 7-Aminoisoquinolones[a]

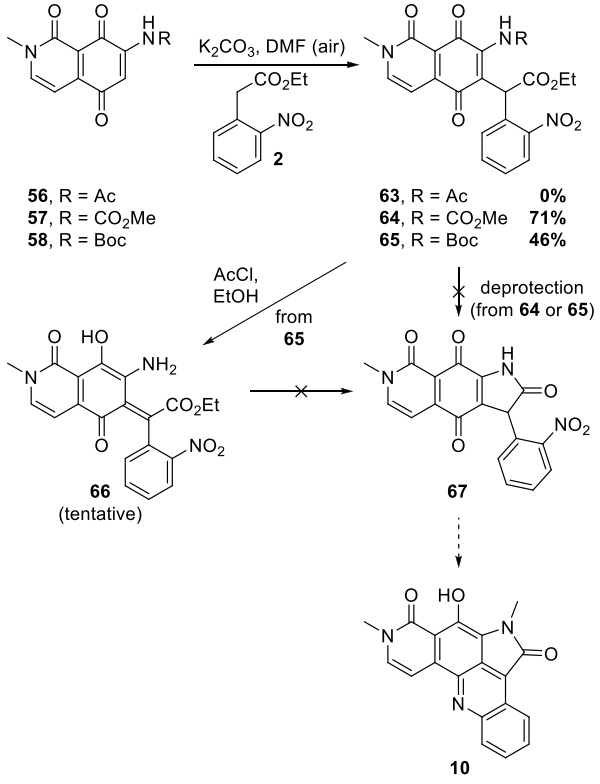


[a]The red double-headed arrows indicate HMBC correlations, which confirmed the regiochemistry of the nitration reaction.

Scheme 9. Oxidations of 7-Aminoisoquinolone 45 and Derivatives



Scheme 10. Michael Addition to 7-Nitrogenated Isoquinolinetriones and Subsequent Transformations



lactam **67**, producing a complex mixture instead. More effort was devoted to the deprotection/cyclization of **65**. Surprisingly, the *t*-butyl carbamate **65** was resistant to deprotection with TFA at rt and gave complex mixtures with $BF_3{\cdot}OEt_2$[19] or H_2SO_4 in dioxane.[20] Treatment with 5 M hydrochloric acid under reflux, or ethanolic HCl, gave a major product that could not be conclusively identified. The ^1H NMR spectrum of this product is consistent with the *ortho*-quinone methide **66** or its geometric isomer, although definitive structural elucidation was precluded by material availability, and hence, this assignment is tentative. Unfortunately, attempts to cyclize this product to give lactam **67**, or a tautomer, were unsuccessful.

CONCLUSIONS

Michael additions of ethyl *o*-nitrophenylacetate (**2**) to quinonoid electrophiles have been investigated as a means to connect fragments comprising the CE ring system and A ring of the marine pyrroloacridine alkaloid alpkinidine (**10**). While the reaction of the anion derived from **2** with isoquinolinetrione **15** proceeded with the wrong regiochemistry, the successful coupling of the two fragments and oxidation to give

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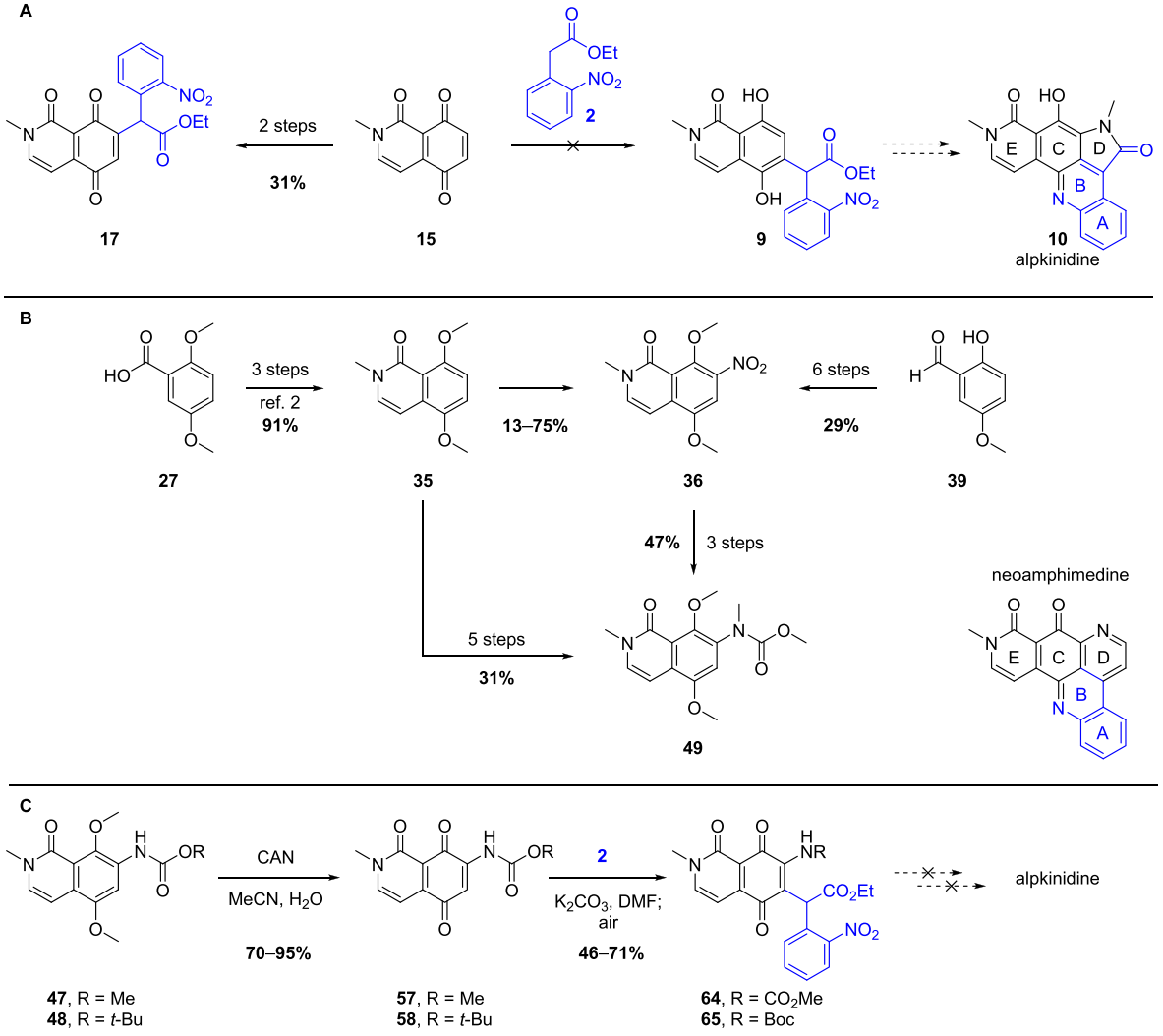
Scheme 11. (A–C) Summary of Major Outcomes of the Current Work



quinone **17** (Scheme 11A) encouraged further exploration of this strategy.

To direct the regiochemistry of the key Michael addition, 7-nitrogentated isoquionlinetriones were targeted, and several efficient syntheses were developed (Scheme 11B). These isoquinolones and the general concept of Michael additions to such synthons may be of value in the synthesis of analogues of the closely related marine alkaloid neoamphimedine, which has a near-identical ABCE ring system to alpkinidine and promising anticancer potential.[3,21−23]

Proof of concept was achieved in that **2** underwent Michael addition to isquinolinetriones **57**/**58** and aerial oxidation to afford adducts **64**/**65**, containing the complete carbon skeleton of alpkinidine. However, attempts to close rings B and D were unsuccessful. The key to bringing the approach outlined herein to fruition probably lies in finding the right order of redox and deprotection reactions on an advanced intermediate like **64**/**65**, an objective that will be pursued in the future.

EXPERIMENTAL SECTION

General. General experimental details are as reported previously.[1,24]

Synthesis. *Ethyl 2-(5,8-Dihydroxy-2-methyl-1-oxo-1,2-dihydroisoquinolin-7-yl)-2-(2-nitrophenyl)acetate (**16**).* Isoquinolinetrione **15**[2] (96 mg, 0.51 mmol) was added to a stirred suspension of ethyl 2-nitrophenylacetate (**2**) (0.25 g, 1.2 mmol) and K_2CO_3 (0.17 g, 1.2 mmol) in DMF (20 mL) at 40 °C. After 1.5 h, the reaction was cooled, diluted with 1 M HCl (30 mL), and extracted with EtOAc (3 × 20 mL). The organic componenets were dried and evaporated, and the crude product was subjected to flash chromotography. Elution with 2:3 EtOAc/hexanes gave **16** (70 mg, 35%) as a pale-yellow oil. R_f (3:2 EtOAc/hexanes) 0.2. IR (ATR) ν_{max} cm^{-1}: 3400−2900 (OH), 1730 (C═O), 1652 (C═O). ^1H NMR (600 MHz) δ 12.7 (s, 1H, OH), 8.04 (dd, J_1 = 7.8, J_2 = 1.2 Hz, 1H, H3″ or H6″), 7.43 (ddd, J_1 = J_2 = 7.8, J_3 = 1.2 Hz, 1H, H4″ or H5″), 7.40 (pseudo ddd [app. t], J = 7.8 Hz, 1H, H4″ or H5″), 7.18 (pseudo dd [app. d], J = 7.8 Hz, 1H, H3″ or H6″), 6.99 (d, J = 7.2 Hz, 1H, H3′ or H4′), 6.91 (s, 1H, H6′), 6.79 (d, J = 7.2 Hz, 1H, H3′ or H4′), 6.02 (s, 1H, H2), 5.39 (s, 1H, OH), 4.27 (m, 1H, O**CH**$_2$CH$_3$), 4.21 (m, 1H, O**CH**$_2$CH$_3$), 3.56 (s, 3H, OMe), 1.26 (t, J = 7.2 Hz, 3H, OCH$_2$**CH**$_3$). ^{13}C NMR (150 MHz) δ 172.3 (C1), 165.5 (C1′), 152.6 (C5′ or C8′), 149.1 (C5′ or C8′), 141.9 (C2″), 133.2 (C1″ or C7′), 133.1 (ArH), 131.4 (ArH), 131.1 (ArH), 128.2 (ArH), 126.6 (C4a′ or C8a′), 125.1 (ArH), 120.1 (C1″ or C7′), 119.4 (ArH), 112.3 (C4a′ or C8a′), 102.4 (ArH), 61.8 (O**CH**$_2$CH$_3$), 47.2 (C2),

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36.5 (NMe), 14.1 ($OCH_2\mathbf{C}H_3$). HRMS (APCI): calcd for $C_{20}H_{19}N_2O_7^+$ $[M + H]^+$ 399.1194; found 399.1187.

*Ethyl 2-(2-Methyl-1,5,8-trioxo-1,2,5,8-tetrahydroisoquinolin-7-yl)-2-(2-nitrophenyl)acetate (**17**).* Ag_2O (0.31 g, 1.34 mmol) was added to a stirred suspension of hydroquinone **16** (70 mg, 0.17 mmol) and $MgSO_4$ (0.56 g, 4.65 mmol) in Et_2O (15 mL) and DME (5 mL). After 16 h, the reaction was filtered through a plug of Celite and washed with DCM (3 × 10 mL). The volatiles were then removed to give **17** as a red-orange oil (61 mg, 88%). R_f (EtOAc) 0.1. IR (ATR) ν_{max} cm^{-1}: 1732 (C═O), 1686 (C═O), 1671 (C═O). ^1H NMR (600 MHz) δ 8.08 (dd, J_1 = 8.4, J_2 = 1.2 Hz, 1H, H3″ or H6″), 7.80 (d, J = 6.6 Hz, 1H, H3 or H4), 7.65 (dt, J_1 = 7.2, J_2 = 1.2 Hz, 1H, H4″ or H5″), 7.53 (dt, J_1 = 7.8, J_2 1.8 Hz, 1H, H4″ or H5″), 7.46 (dd, J_1 = 7.8, J_2 = 1.2 Hz, 1H, H3″ or H6″), 6.74 (d, J = 6.6 Hz, 1H, H3′ or H4′), 6.40 (d, J = 1.2 Hz, 1H, H6′), 5.80 (s, 1H, H2), 4.25 (m, 2H, $O\mathbf{C}H_2CH_3$), 4.20 (m, 2H, $O\mathbf{C}H_2CH_3$), 3.66 (s, 3H, NMe), 1.25 (t, J 7.2 Hz, 3H, $OCH_2\mathbf{C}H_3$). ^{13}C NMR (150 MHz) δ 184.8 (C5′ or C8′), 181.2 (C5′ or C8′), 169.7 (C1), 158.4 (C1′), 149.5 (C2″), 149.1 (C1″ or C7′), 145.5 (ArH), 143.3 (C1″ or C7′), 133.8 (ArH). 133.0 (ArH), 131.1 (ArH), 130.0 (ArH), 129.4 (C4a′ or C8a′), 125.9 (ArH), 119.3 (C4a′ or C8a′), 100.3 (ArH), 62.4 ($O\mathbf{C}H_2CH_3$), 47.9 (C2), 39.2 (NMe), 14.1 ($OCH_2\mathbf{C}H_3$).

*N-(2,2-Diethoxyethyl)-2,5-diacetoxybenzamide (**22**) and N-(2,2-Diethoxyethyl)-2-hydroxy-5-acetoxybenzamide (**23**).* A solution of 2,5-diacetoxybenzoic acid (**20**)[7] (5.02 g, 21.1 mmol) and $SOCl_2$ (10 mL, 0.14 mol) in PhMe (25 mL) was heated under reflux for 2 h before the solvent and excess $SOCl_2$ were removed by distillation. The residue was cooled to 0 °C, and a solution of NEt_3 (10 mL, 72 mmol) in PhMe (10 mL) was added dropwise, followed by a solution of 2,2-diethoxyethanamine (**21**) (3.4 g, 23 mmol) in PhMe (10 mL) dropwise. The mixture was stirred at rt for another 3 h before being diluted with H_2O (50 mL) and extracted with EtOAc (3 × 20 mL). The extract was washed with sat. aq. $NaHCO_3$ (20 mL), dried, and evaporated, and the residue was subjected to flash chromatography. Elution with 2:3 EtOAc/hexanes gave **22** (1.64 g, 22%) as a pale-yellow oil. R_f (3:2 EtOAc/hexanes) 0.35. IR (ATR) ν_{max} cm^{-1}: 2977 (NH), 1764 (Ac C═O), 1661 (C═O). ^1H NMR (600 MHz) δ 7.59 (d, J = 2.4 Hz, 1H, H6), 7.18 (dd, J_1 = 9.0, J_2 = 2.4 Hz, 1H, H4), 7.11 (d, J = 9.0 Hz, 1H, H3), 6.76 (s, 1H, NH), 4.58 (t, J = 5.4 Hz, 1H, H2′), 3.74−3.66 (m, 2H, $O\mathbf{C}H_2CH_3$), 3.60−3.52 (m, 4H, $O\mathbf{C}H_2CH_3$ and H1′), 2.34 (s, 3H, OMe), 2.28 (s, 3H, OMe), 1.21 (t, J = 7.2 Hz, 6H, $OCH_2\mathbf{C}H_3$). ^{13}C NMR (150 MHz) δ 169.2 (C═O), 168.8 (C═O), 164.4 (C═O), 148.3 (ArO), 145.4 (ArO), 128.7 (C1), 125.2 (ArH), 124.4 (ArH), 123.5 (ArH), 100.5 (C2′), 62.9 ($O\mathbf{C}H_2CH_3$), 42.5 (C1′), 21.1 (2 × $CO\mathbf{C}H_3$), 15.4 ($OCH_2\mathbf{C}H_3$). HRMS (APCI): calcd for $C_{17}H_{24}NO_7^+$ $[M + H]^+$ 354.1547; found 354.1570.

Further elution with 3:2 EtOAc/hexanes gave **23** (2.52 g, 38%) as a pale-yellow oil. IR (ATR) ν_{max} cm^{-1}: 3600−2800 (OH), 2977 (NH), 1760 (Ac C═O), 1646 (C═O). ^1H NMR (600 MHz) δ 12.1 (s, 1H, OH), 7.14−7.08 (m, 2H, H4 and H6), 6.96 (d, J = 8.4 Hz, 1H, H3), 6.51 (s, 1H, NH), 4.60 (t, J = 5.0 Hz, 1H, H2′), 3.78−3.68 (m, 2H, $O\mathbf{C}H_2CH_3$), 3.61−3.53 (m, 4H, $O\mathbf{C}H_2CH_3$ and C1′), 2.28 (s, 3H, $CO\mathbf{C}H_3$), 1.23 (t, J = 7.0 Hz, 6H, $OCH_2\mathbf{C}H_3$). ^{13}C NMR (150 MHz) δ 169.6 (C═O), 169.2 (C═O), 159.2 (C2), 142.0 (C4), 127.6 (ArH), 119.3 (ArH), 118.0 (ArH), 114.0 (C1), 100.4 (C2′), 63.1 ($O\mathbf{C}H_2CH_3$), 42.0 (C1′), 20.9 ($CO\mathbf{C}H_3$), 15.3 ($OCH_2\mathbf{C}H_3$). HRMS (APCI): calcd for $C_{15}H_{22}NO_6^+$ $[M + H]^+$ 312.1451; found 312.1442.

*N-(2,2-Diethoxyethyl)-2,5-dihydroxybenzamide (**25**).* Methyl 2,5-dihydroxybenzoate (**24**) (1.97 g, 11.8 mmol) was heated with aminoacetal **21** (5.0 mL, 35 mmol) at 100 °C under $CaCl_2$ guard for 24 h before being diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The organic extract was dried and evaporated to give **25** (2.07 g, 65%) as a pale-yellow oil. R_f (2:3 EtOAc/hexanes) 0.1. IR (ATR) ν_{max} cm^{-1}: 3600−2800 (OH), 2978 (NH), 1640 (C═O). ^1H NMR (600 MHz) δ 11.5 (s, 1H, OH), 7.42 (br s, 1H, OH), 7.31 (s, 1H, NH), 7.07 (d, J = 2.4 Hz, 1H, H6), 6.88 (dd, J_1 = 8.4, J_2 = 2.4 Hz, 1H, H4), 6.78 (d, J = 8.4 Hz, 1H, H3), 4.66 (t, J = 4.8 Hz, 1H, H2′), 3.73 (m, 2H, $O\mathbf{C}H_2CH_3$), 3.55 (m, 4H, $O\mathbf{C}H_2CH_3$ and C1′), 1.20 (t, J = 7.2 Hz, 6H, $OCH_2\mathbf{C}H_3$). ^{13}C NMR (150 MHz) δ 169.8 (C═O), 154.1 (C2), 148.4 (C5), 122.3 (ArH), 118.9 (ArH), 114.6 (ArH), 112.2 (C1), 100.8 (C2′), 63.5 ($O\mathbf{C}H_2CH_3$), 42.3 (C1′), 15.2 ($OCH_2\mathbf{C}H_3$). HRMS (ESI^-): calcd for $C_{13}H_{18}NO_5^-$ $[M-H]^-$ 268.1167; found 268.1190.

*5,8-Dihydroxyisoquinolin-1(2H)-one (**26**).* Method 1: Concentrated H_2SO_4 (5 mL) was added dropwise to neat **25** with stirring at 0 °C under $CaCl_2$ guard. After the addition was complete, the solution was allowed to warm to rt, then stirred at 50 °C for 24 h. The reaction was diluted with H_2O (20 mL) and carefully neutralized with ice cold sat. aq. $NaHCO_3$ (∼30 mL) until effervescing ceased, then extracted with EtOAc (3 × 20 mL). The extract was dried and evaporated, and the crude product was subjected to flash chromatography. Elution with 2:3 EtOAc/hexanes gave isoquinolone **26** as an off-white solid (77 mg, 6%), mp 260−263 °C. R_f (2:3 EtOAc/hexanes) 0.1. IR (ATR) ν_{max} cm^{-1}: 3500−2700 (OH), 2886 (NH), 1639 (C═O). ^1H NMR (600 MHz, DMSO-d_6) δ 12.3 (s, 1H, OH), 11.6 (s, 1H, NH), 9.44 (s, 1H, OH), 7.09 (d, J = 7.2 Hz, 1H, H3), 7.02 (d, J = 9.0 Hz, 1H, H6 or H7), 6.72 (d, J = 7.2 Hz, 1H, H4), 6.62 (d, J = 9.0 Hz, 1H, H6 or H7). ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.9 (C1), 153.0 (C5 or C8), 143.6 (C5 or C8), 126.9 (ArH), 126.8 (C4a or C8a), 119.1 (ArH), 111.6 (C4a or C8a), 111.4 (ArH), 101.7 (ArH). HRMS (ESI^-): calcd for $C_9H_6NO_3^-$ $[M-H]^-$ 176.0340; found 176.0353.

Method 2: 5,8-Dimethoxyisoquinolin-1(2*H*)-one (**29**) (0.16 g, 0.76 mmol) was added to pyridine hydrochloride,[2] and the mixture was heated under reflux for 20 min before being cooled and diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated to give hydroquinone **26** as an off-white solid (0.13 g, 95%), identical to the material described above.

*N-(2,2-Diethoxyethyl)-2,5-dimethoxybenzamide (**28**).* A solution of 2,5-dimethoxybenzoic acid (**27**) (0.68 g, 3.7 mmol) and $SOCl_2$ (5.0 mL, 69 mmol) in PhMe (15 mL) was heated under reflux for 2 h before the solvent and excess $SOCl_2$ were removed by distillation. The residue was cooled to 0 °C, and a solution of NEt_3 (5.0 mL, 36 mmol) in PhMe (5 mL) was added dropwise, followed by a solution of aminoacetal **21** (3.4 g, 23 mmol) in PhMe (5 mL) dropwise. The mixture was stirred at rt for another 3 h before being diluted with H_2O (30 mL) and extracted with EtOAc (3 × 20 mL). The extract was washed with sat. aq. $NaHCO_3$ (20 mL), dried, and evaporated, and the crude product was subjected to flash chromatography. Elution with 1:4 EtOAc/hexanes gave **28** (0.98 g, 88%) as an amber oil. R_f (2:3 EtOAc/hexanes) 0.2. IR (ATR) ν_{max} cm^{-1}: 2975 (NH), 1652 (C═O). ^1H NMR (500 MHz) δ 8.23 (s, 1H, NH), 7.72 (d, J = 3.5 Hz, 1H, H6), 6.94 (dd, J_1 = 9.0, J_2 =

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3.5 Hz, 1H, H4), 6.86 (d, J = 9.0 Hz, 1H, H3), 4.59 (t, J = 5.5 Hz, 1H, H2′), 3.87 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.71 (dq, J_1 = J_2 = 7.0 Hz, 2H, O**CH$_2$**CH$_3$), 3.60−3.50 (m, 4H, O**CH$_2$**CH$_3$ and H1′), 1.20 (t, J = 7.0 Hz, 6H, OCH$_2$**CH$_3$**). ^{13}C NMR (125 MHz) δ 165.1 (C═O), 153.9 (ArO), 151.9 (ArO), 121.9 (C1), 119.3 (ArH), 115.6 (ArH), 113.1 (ArH), 101.0 (C2′), 62.8 (O**CH$_2$**CH$_3$), 56.9 (OMe), 55.8 (OMe), 42.4 (C1′), 15.4 (OCH$_2$**CH$_3$**). HRMS (ESI): calcd for $C_{17}H_{26}N_2NaO_5^+$ [M + Na + MeCN]$^+$ 361.1741; found 361.1734.

*5,8-Dimethoxyisoquinolin-1(2H)-one (**29**).* Concentrated H_2SO_4 (10 mL) was added dropwise to neat **28** (0.901 g, 3.03 mmol) with stirring at 0 °C. After the addition was complete, the solution was allowed to warm to rt, then stirred at 50 °C under $CaCl_2$ guard. After 24 h, the solution was cooled and carefully neutralized with ice-cold sat. aq. $NaHCO_3$ (∼50 mL) until effervescing ceased, then extracted with EtOAc (3 × 30 mL). The extract was dried and evaporated to give an off-white solid (0.371 g, note: the yield was affected by a spill), which crystallized from DCM/hexanes affording isoquinolone **29** as colorless plates (0.155 g, 25%), identical to the material reported previously.[25]

*5,8-Dimethoxy-2-methyl-7-nitroisoquinolin-1(2H)-one (**36**) and 5,8-Dimethoxy-2-methyl-4-nitroisoquinolin-1(2H)-one (**37**)[a].* Method A: Concentrated HNO_3 (1.0 mL, 17 mmol) was added dropwise to a suspension of **35**[2] (91 mg, 0.42 mmol) and Ag_2O (0.53 g, 2.29 mmol) in acetone (15 mL) at 0 °C. After 1 h, the reaction was diluted with H_2O (20 mL) and sat. aq. $NaHCO_3$ (20 mL), then extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 3:2 EtOAc/hexanes gave **36** as yellow rods (83 mg, 75%), mp 196−200 °C. R_f (3:2 EtOAc/hexanes) 0.25. IR (ATR) ν_{max} cm^{-1}: 1651 (C═O). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.70 (d, J = 7.2 Hz, 1H, H3), 7.61 (s, 1H, H6), 6.71 (d, J = 7.2 Hz, 1H, H4), 3.94 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.49 (s, 3H, NMe). ^{13}C NMR (150 MHz, DMSO-d_6) δ 158.6 (C1), 149.6 (C5 or C8), 146.4 (C5 or C8), 141.9 (C7), 137.3 (ArH), 133.7 (C4a or C8a), 119.4 (C4a or C8a), 106.7 (ArH), 97.6 (ArH), 63.6 (OMe), 56.7 (OMe), 37.0 (NMe). HRMS (APCI): calcd for $C_{12}H_{13}N_2O_5^+$ [M + H]$^+$ 265.0819; found 265.0824.

Further elution gave **37** as yellow plates (15 mg, 14%), mp 177−180 °C. R_f (3:2 EtOAc/hexanes) 0.1. IR (ATR) ν_{max} cm^{-1}: 1652 (C═O). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.41 (s, 1H, H3), 7.43 (d, J = 9.0 Hz, 1H, H6 or H7), 7.17 (d, J = 9.0 Hz, 1H, H6 or H7), 3.82 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.41 (s, 3H, NMe). ^{13}C NMR (150 MHz, DMSO-d_6) δ 158.4 (C═O), 154.4 (C5 or C8), 146.1 (C5 or C8), 135.0 (C3), 129.1 (C4), 120.7 (C4a or C8a), 117.3 (C6 or C7), 114.2 (C4a or C8a), 112.2 (C6 or C7), 56.7 (OMe), 36.7 (NMe). HRMS (APCI): calcd for $C_{12}H_{13}N_2O_5^+$ [M + H]$^+$ 265.0832; found 265.0819.

Method B: A solution of concentrated HNO_3 (1.0 mL, 17 mmol) in acetone (10 mL) was added to a stirred solution of **35**[2] (1.13 g, 5.17 mmol) in AcOH (10 mL) and acetone (10 mL). After 1 h, the mixture was diluted with H_2O (30 mL) and sat. aq. $NaHCO_3$ (20 mL), then extracted with EtOAc (3 × 20 mL). The extract was dried and evaporated. Precipitation from a mixture of MeOH/DCM/hexanes gave **36** as a yellow solid (0.96 g, 70%), identical to the material described above.

Method C (**36** exclusively): Ice-cold conc. H_2SO_4 (10 mL) was added dropwise to neat **44** (5.37 g, 15.1 mmol) with stirring at 0 °C. After the addition was complete, the solution was warmed to rt, then heated to 100 °C. After 1 h, the solution was cooled and carefully neutralized with ice-cold sat. aq. $NaHCO_3$ until effervescing ceased. The aqueous phase was extracted with EtOAc (3 × 30 mL), and the extract was dried and evaporated. The residue was purified by flash column chromatography. Elution with 3:2 EtOAc/hexanes yielded isoquinolone **36** as a yellow solid (2.59 g, 65%), identical to the material described above.

*2-Hydroxy-5-methoxybenzoic acid (**39**).* Method A:[9] Anhydrous THF (250 mL) was added to anhydrous $MgCl_2$ (15.35 g, 161.2 mmol) and paraformaldehyde (7.26 g, 242 mmol) under a positive pressure of N_2. NEt_3 (22.47 mL, 161.2 mmol) was added dropwise to the stirred suspension, and after 10 min, 4-methoxyphenol (**38**) (10.00 g, 80.55 mmol) was added, resulting in an opaque, light-green mixture. The reaction mixture was heated under gentle reflux whereupon it rapidly turned orange/yellow color. After 6 h, the reaction mixture was cooled to rt and rinsed with ether (150 mL) into a separatory funnel. The organic phase was washed with 1 M HCl (3 × 150 mL), water (3 × 150 mL), and brine (150 mL), dried, and evaporated to leave a pale-yellow oil, which was subjected to flash column chromatography. Elution with 2:23 EtOAc/hexanes gave the benzaldehyde **39** as a pale-yellow oil (12.15 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.65 (s, 1H), 9.86 (d, J = 0.6 Hz, 1H, CHO), 7.14 (dd, J = 3.2, 9.0 Hz, 1H, H4), 7.00 (d, J = 3.2 Hz, 1H, H6), 6.93 (d, J = 9.0 Hz, 1H, H3), 3.82 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.3 (CO), 156.2 (ArO), 152.9 (ArO), 124.4 (ArH), 119.2, 117.9 (ArH), 114.3 (ArH), 56.1 (OMe). The NMR data are consistent with the literature.[26]

Method B:[10] Anhydrous $AlCl_3$ (12.04 g, 90.32 mmol) was added to a stirred solution of 2,5-dimethoxybenzaldehyde (10.00 g, 60.12 mmol) in anhydrous DCM (200 mL) at 0 °C under N_2. The reaction mixture was allowed to slowly warm to rt, and stirring was continued for 5 h. When TLC indicated that the reaction was complete, the mixture was diluted with ice/water (300 mL) and the DCM layer was separated. The aqueous phase was extracted with EtOAc (2 × 100 mL). The aqueous phase was then acidified with concentrated HCl and reextracted with EtOAc (2 × 100 mL). The combined organic phase was washed with brine (200 mL), dried, and evaporated to give a brown oil that was subjected to flash column chromatography. Elution with 8:92 EtOAc/hexanes gave the phenol **39** as a colorless oil (7.84 g, 86%), spectroscopically identical with the material described above.

*2-Hydroxy-5-methoxy-3-nitrobenzaldehyde (**40**).* A solution of 70% HNO_3 (5.25 mL, 33.9 mmol) in AcOH (19 mL) was added dropwise to a cooled, stirred solution of the benzaldehyde **39** (12.15 g, 79.86 mmol) in AcOH (120 mL) at such a rate as to maintain the temperature between 10 and 15 °C (50 min). After stirring for a further 45 min, water (60 mL) was added and the precipitated solid was collected by vacuum filtration, washed with water, and air-dried to afford to give **40** as a yellow solid (11.80 g, 75%), mp 132−133 °C [lit.[11] 132−133 °C]. R_f (1:9 EtOAc/hexanes) 0.4. IR (ATR) ν_{max} cm^{-1}: 3200−2800 (OH), 1691 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1H, OH), 10.43 (s, 1H, CHO) 7.84 (d, J = 3.3 Hz, 1H, H4 or H6), 7.70 (d, J = 3.3 Hz, 1H, H4 or H6), 3.87 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.3 (C═O), 152.2 (ArO), 151.3 (ArO), 126.3 (C3), 123.1 (C4 or C6), 117.1 (C1), 115.3 (C4 or C6), 56.5 (OMe). The NMR data are consistent with the literature.[11]

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*2,5-Dimethoxy-3-nitrobenzaldehyde (**41**).* Anhydrous K_2CO_3 (13.68 g, 99 mmol) and MeI (6.19 mL, 99.3 mmol) were added to a solution of **40** (9.75 g, 49.5 mmol) in dry DMF (110 mL) in a stoppered flask. The mixture was stirred at 60 °C for 12 h, then poured onto ice/water (1 L), and extracted with EtOAc (3 × 200 mL). The extract was washed with water (10 × 100 mL) and brine (150 mL), dried, and evaporated to give the dimethyl ether **41** as a yellow solid (9.50 g, 90%), mp 111−113 °C [lit.[27] 113 °C]. R_f (1:9 EtOAc/hexanes) 0.3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H, CHO), 7.62 (d, J = 3.3 Hz, 1H, H4 or H6), 7.57 (d, J = 3.3 Hz, 1H, H4 or H6), 4.03 (s, 3H, OMe), 3.88 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.6 (C═O), 155.6 (C2), 150.2 (C5), 131.9 (C3), 117.3 (C4 or C6), 117.0 (C4 or C6), 65.9 (2-OMe), 56.5 (5-OMe). The C1 signal was not observed/coincident. The NMR data are consistent with the literature.[28]

*2,5-Dimethoxy-3-nitrobenzoic acid (**42**).* A solution of $KMnO_4$ (10.7 g, 67.7 mmol) in water (81 mL) was added to a mixture of 2,5-dimethoxy-3-nitrobenzaldehyde (**41**) (9.5 g, 45 mmol) and $KHCO_3$ (9.0 g, 90 mmol) in boiling water (130 mL). When TLC indicated that the reaction was complete, the hot solution was filtered through a pad of Celite, washed through with water (100 mL), and allowed to cool. The reddish yellow filtrate was acidified with conc. HCl, and the resulting precipitate was collected by vacuum filtration, washed with water, and air-dried to give benzoic acid **42** as a pale-yellow solid (8.19 g, 80%), mp 155−160 °C [lit.[29] 181−183 °C]. ^{1}H NMR (400 MHz, DMSO), δ = 7.66 (d, J = 3.3 Hz, 1H, H4 or H6), 7.51 (d, J = 3.3 Hz, H4 or H6, 1H), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe). ^{13}C NMR (100 MHz, DMSO) δ 165.4 (C═O), 154.5 (C2), 145.6 (C5 or C3), 144.6 (C5 or C3), 129.0 (C1), 120.1 (ArH), 112.5 (ArH), 63.9 (2-OMe), 56.4 (5-OMe). The NMR data are consistent with the literature.[30]

*N-(2,2-Diethoxyethyl)-2,5-dimethoxy-N-methyl-3-nitrobenzamide (**44**).* A solution of 2,5-dimethoxy-3-nitrobenzoic acid (**42**) (7.59 g, 33.4 mmol) and $SOCl_2$ (10.67 mL, 147.1 mmol) in DCM (10 mL) was heated under reflux and moisture guard for 2 h before the solvent and excess $SOCl_2$ were evaporated under a stream of N_2. The residue was cooled to 0 °C, and a solution of pyridine (10.66 mL, 132.3 mmol) in DCM (7 mL) was added dropwise, followed by dropwise addition of a solution of amine **43**[2] (7.38 g, 50.2 mmol) in DCM (7 mL). The mixture was stirred at rt for 3 h, then diluted with H_2O (50 mL), and extracted with DCM (3 × 20 mL). The extract was washed with 10% aq. $CuSO_4$ (4 × 20 mL), sat. aq. $NaHCO_3$ (20 mL), and brine and dried and evaporated to give the amide **44** as a reddish oil (9.50 g, 80%) sufficiently pure for the next step. R_f (1:19 MeOH/DCM) 0.35. IR (ATR) ν_{max} cm^{-1}: 1691 (C═O). ^{1}H NMR (500 MHz, $CDCl_3$; an 18*:10$^{\#}$ mixture of rotamers) δ 7.36* (d, J = 3.2 Hz, 1H, H4 or H6), 7.34$^{\#}$ (d, J = 3.2 Hz, 1H, H4 or H6), 7.05$^{\#}$ (d, J = 3.1 Hz, 1H, H4 or H6), 7.00* (d, J = 3.1 Hz, 1H, H4 or H6), 4.79* (t, J = 5.5 Hz, 1H, H2′), 4.48$^{\#}$ (t, J = 5.3 Hz, 1H, H2′), 3.89* (s, 3H, OMe), 3.87$^{\#}$ (s, 3H, OMe), 3.83* (s, 3H, OMe), 3.82$^{\#}$ (s, 3H, OMe), 3.81−3.69$^{\#}$ (m, 4H, OCH_2), 3.66−3.46* (m, 4H, OCH_2), 3.45−3.32* (m, 2H, H1′), 3.30−3.22$^{\#}$ (m, 2H, H1′), 3.18$^{\#}$ (s, 3H, NMe), 2.92* (s, 3H, NMe), 1.24* (t, J = 7.0 Hz, 6H, 2 × CH_3), 1.17$^{\#}$ (t, J = 7.0 Hz, 3H, CH_3), 1.12$^{\#}$ (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6$^{\#}$ (C═O), 167.4* (C═O), 155.6* (C2), 155.5$^{\#}$ (C2), 144.3*, 144.2$^{\#}$, 143.3*, 143.0$^{\#}$ 135.2*, 135.0$^{\#}$, 119.4$^{\#}$ (C4 or C6), 118.6* (C4 or C6), 110.6*$^{\#}$ (C4 or C6), 101.4$^{\#}$ (CHO_2), 101.0* (CHO_2), 64.0* (OMe or OCH_2), 63.9* OMe or OCH_2), 63.8$^{\#}$ (OMe or OCH_2), 63.5$^{\#}$ (OMe or OCH_2), 63.4*$^{\#}$ (OMe or OCH_2), 56.31* (C5-OMe), 56.27$^{\#}$ (C5-OMe), 53.8$^{\#}$ (NCH_2), 50.9* (NCH_2), 38.5* (NMe), 34.7$^{\#}$ (NMe), 15.5* (2 × CH_3), 15.4$^{\#}$ (CH_3), 15.3$^{\#}$ (CH_3). HRMS (APCI): calcd for $C_{16}H_{25}N_2O_7^+$ $[M + H]^+$ 357.1656; found 357.1750.

*7-Amino-5,8-dimethoxy-2-methylisoquinolin-1(2H)-one (**45**).* Method A: Iron powder (2.01 g, 35.4 mmol) was added to a vigorously stirred solution of **36** (2.32 g, 8.78 mmol) in AcOH (30 mL), H_2O (30 mL), and MeOH (15 mL). After 1.5 h, the reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 25 mL). The extract was washed with sat. aq. $NaHCO_3$ (3 × 20 mL), dried, and evaporated, and the crude product was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave aniline **45** as an amber oil (1.65 g, 80%). R_f (1:19 MeOH/DCM) 0.2. IR (ATR) ν_{max} cm^{-1}: 3337 (NH_2), 1646 (C═O). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.04 (dd, J = 7.2, 0.6 Hz, 1H, H3), 6.73 (s, 1H, H6), 6.50 (dd, J = 7.2, 0.6 Hz, 1H, H4), 5.21 (s, 2H, NH_2), 3.79 (s, 3H, OMe), 3.60 (s, 3H, OMe), 3.39 (s, 3H, NMe). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.2 (C1), 150.2 (C5), 140.9 (C8), 136.9 (C7), 128.5 (C3), 119.9 (C4a or C8a), 118.7 (C4a or C8a), 102.0 (C4 or C6), 98.8 (C4 or C6), 60.1 (C8-OMe), 55.6 (C5-OMe), 36.4 (NMe). HRMS (APCI): calcd for $C_{12}H_{15}N_2O_3^+$ $[M + H]^+$ 235.1081; found 235.1077.

Method B: $SnCl_2{\cdot}2H_2O$ (5.64 g, 25.0 mmol) was added to a solution of **36** (1.32 g, 5.00 mmol) in EtOH (30 mL), and the mixture was heated under reflux for 1.5 h. The reaction mixture was cooled to rt, then poured into ice (150 g), and the resulting suspension was made alkaline by an addition of sat. aq. $NaHCO_3$. The aqueous phase was extracted with DCM (3 × 25 mL), and the extract was washed with brine (25 mL) and evaporated. The residue was then acidified with 1 M HCl and extracted with DCM (2 × 25 mL). The aqueous layer was made alkaline by addition of solid $NaHCO_3$. The excess solid was filtered, and the filtrate was extracted with DCM (3 × 20 mL). The combined organic phase was washed with brine (20 mL) and evaporated to afford the aniline **45** as a yellow oil (1.00 g, 85%), spectroscopically identical to the material described above.

*7-Acetylamino-5,8-dimethoxy-2-methylisoquinolin-1(2H)-one (**46**).* Ac_2O (0.50 mL, 2.7 mmol) was added to a stirred solution of **45** (0.21 g, 0.89 mmol) and NEt_3 (0.50 mL, 3.5 mmol) in DCM (15 mL). After 24 h, the reaction mixture was diluted with H_2O (30 mL) and extracted with DCM (3 × 20 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:99 MeOH/DCM gave acetamide **46** as a pale-yellow solid (0.22 g, 88%), mp 177−180 °C. R_f (1:19 MeOH/DCM) 0.3. IR (ATR) ν_{max} cm^{-1}: 3321 (NH), 1674 (O═CNH), 1649 (O═C1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.47 (s, 1H, NH), 8.05 (s, 1H, H6), 7.35 (d, J = 7.2 Hz, 1H, H3), 6.61 (d, J = 7.2 Hz, 1H, H4), 3.83 (OMe), 3.71 (OMe), 3.44 (NMe), 2.16 (CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.2 (HNC═O), 159.0 (C1), 149.2 (C5 or C8), 142.4 (C5 or C8), 132.5 (C3 or C6), 130.6 (C3 or C6), 125.3 (C7), 119.0 (C4a or C8a), 107.3 (C4a or C8a), 98.1 (C4), 61.6 (C8-OMe), 55.9 (C5-OMe), 36.6 (NMe), 24.0 (CH_3). HRMS (APCI): calcd for $C_{14}H_{17}N_2O_4^+$ $[M + H]^+$ 277.1183; found 277.1176.

*5,8-Dimethoxy-7-methoxycarbonylamino-2-methylisoquinolin-1(2H)-one (**47**).* Methyl chloroformate (0.10 mL, 1.3 mmol) was added to a stirred solution of aniline **45** (65 mg,

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0.28 mmol) and NEt_3 (0.12 mL, 0.86 mmol) in DCM (10 mL) at 0 °C. The solution was allowed to warm to rt, and stirring was continued for 3 h; then, the reaction was diluted with H_2O (20 mL) and extracted with DCM (3 × 20 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with EtOAc gave carbamate **47** as a pale-yellow solid (54 mg, 66%), mp 160−163 °C. R_f (EtOAc) 0.3. IR (ATR) ν_{max} cm^{-1}: 3423 (NH), 1736 (O═COMe), 1650 (O═C1). ^1H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H, NH), 7.60 (s, 1H, H6), 6.97 (d, J = 7.2 Hz, 1H, H3), 6.77 (d, J = 7.2 Hz, 1H, H4), 3.94 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.55 (s, 3H, NMe). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.3 (C1), 154.3 (CO_2Me), 150.8 (C5), 140.4 (C8), 130.8 (C3 + C6 coincident), 124.9 (C7), 119.7 (C4a or C8a), 103.8 (C4a or C8a), 100.0 (C4), 62.4 (C8-OMe), 56.1 (C5-OMe), 52.5 (CO_2Me), 37.5 (NMe). HRMS (APCI): calcd for $C_{14}H_{17}N_2O_5^+$ $[M + H]^+$ 293.1132; found 293.1148.

*7-t-Butoxycarbonylamino-5,8-dimethoxy-2-methylisoquinolin-1(2H)-one (**48**).* A solution of Boc_2O (2.19 g, 10.0 mmol), aniline **45** (1.00 g, 4.27 mmol), and NEt_3 (1.7 mL, 12 mmol) in THF (30 mL) was heated under reflux for 3 h. The solution was cooled, diluted with H_2O (30 mL), and extracted with EtOAc (3 × 20 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 7:3 EtOAc/hexanes gave **48** as a pale-yellow oil (1.11 g, 77%). R_f (7:3 EtOAc/hexanes) 0.3. IR (ATR) ν_{max} cm^{-1}: 3420 (NH) 1722 (O═CO), 1650 (O═C1). ^1H NMR (400 MHz, $CDCl_3$) δ = 8.10 (s, 1H, NH), 7.43 (s, 1H, H6), 6.95 (d, J = 7.5 Hz, 1H, H3), 6.76 (d, J = 7.4 Hz, 1H, H4), 3.94 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.54 (s, 3H, NMe), 1.55 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2 (C1), 152.9 (CO_2), 150.6 (C5), 140.2 (C8), 131.2 (C3 or C6), 130.4 (C3 or C6), 124.4 (C7), 119.5 (C4a or C8a), 103.8 (C4a or C8a), 99.9 (C4), 80.8 (*t*-Bu−O), 62.2 (C8-OMe), 56.0 (C5-OMe), 37.3 (NMe), 28.4 (3 × CH_3). HRMS (APCI): calcd for $C_{17}H_{23}N_2O_5^+$ $[M + H]^+$ 335.1601; found 335.1604.

*5,8-Dimethoxy-7-methoxycarbonyl(methyl)amino-2-methylisoquinolin-1(2H)-one (**49**).* Method A: NaH 60% dispersion in oil (9 mg, 0.2 mmol) and MeI (13 μL, 0.21 mmol) were added to a stirred solution of methyl carbamate **47** (50 mg, 0.17 mmol) in anhydrous THF (5 mL) at 0 °C under N_2. The ice bath was removed, and stirring was continued for 3 h; then, the reaction mixture was poured into water (50 mL) and extracted with DCM (3 × 20 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave tertiary carbamate **49** as a white solid (39 mg, 75%), mp 150−155 °C. R_f (EtOAc) 0.3. IR (ATR) ν_{max} cm^{-1}: 1702 (O═COMe), 1671 (O═C1). ^1H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, J = 6.6 Hz, 1H, H3), 6.80 (s, 1H, H6), 6.73 (d, J = 6.9 Hz, 1H, H4), 3.85 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.61 (s, 3H, OMe), 3.51 (s, 3H, N2Me), 3.23 (s, 3H, C7-NMe). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.3 (C1), 156.6 (CO_2), 150.3 (C5 or C8), 149.7 (C5 or C8), 132.7 (C4 or C6), 130.0 (C4 or C6), 120.8 (C7), 112.5 (C4a or C8a), 107.7 (C4a or C8a), 99.6 (C4), 62.1 (C8-OMe), 56.0 (C5-OMe), 53.0 (CO_2Me), 37.7 (NMe), 37.4 (NMe). HRMS (APCI): calcd for $C_{15}H_{19}N_2O_5^+$ $[M + H]^+$ 307.1304, found 307.1289.

Method B: MeI (0.11 mL, 1.77 mmol) and NaH 60% dispersion in oil (72 mg, 1.8 mmol) were added to a stirred solution of **54** (123 mg, 0.44 mmol) in anhydrous THF (1 mL) under N_2. Stirring was continued overnight, then the reaction mixture was poured into ice/water (30 mL) and extracted with DCM (3 × 10 mL). The extract was washed with brine, dried, and evaporated, and the residue was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave tertiary carbamate **49** as a white solid (106 mg, 78%), identical with the material described above.

*7-t-Butoxycarbonyl(methyl)amino-5,8-dimethoxy-2-methylisoquinolin-1(2H)-one (**50**).* NaH 60% dispersion in oil (140 mg, 3.5 mmol) and MeI (0.22 mL, 3.5 mmol) were added to a stirred solution of **48** (0.78 g, 2.3 mmol) in anhydrous THF (10 mL) at 0 °C under N_2. The ice bath was removed, and stirring was continued for 3 h; then, the reaction mixture was poured into water (100 mL) and extracted with DCM (3 × 30 mL). The extract was washed with brine, dried, and evaporated, and the residue was subjected to flash column chromatography. Elution with 1:19 MeOH/DCM gave tertiary carbamate **50** as a pale-yellow oil (0.75 g, 92%). R_f 0.35 (1:19 MeOH/DCM). IR (ATR) ν_{max} cm^{-1}: 1720 (O═COMe), 1656 (O═C1). ^1H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 7.5 Hz, 1H, H3), 7.10 (s, 1H, H6), 6.64 (d, J = 7.4 Hz, 1H, H4), 3.86 (s, 3H, OMe), 3.67 (s, 3H, OMe), 3.45 (s, 3H, NMe), 3.10 (s, 3H, NMe), 1.31 (br s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, DMSO-d_6), 159.0 (C1), 154.3 (br, CO_2), 149.5 (C8), 148.8 (br, C5), 134.8 (br, C6), 134.0 (C3), 129.0 (br, C7), 119.6 (C4a or C8a), 113.3 (br, C4a or C8a), 97.9 (C4), 79.2 (br, *t*-Bu−O), 61.4 (C8-OMe), 56.2 (C5-OMe), 36.9 (br, 7-NMe) 36.7 (2-NMe), 28.0 (3 × CH_3). One NMe signal was not observed/coincident. HRMS (APCI): calcd for $C_{18}H_{25}N_2O_5^+$ $[M + H]^+$ 349.1758; found 349.1764.

*8-Hydroxy-5-methoxy-2-methylisoquinolin-1(2H)-one (**51**).* Anhydrous $AlCl_3$ (4.56 g, 34.2 mmol) was added to a stirred solution of **35** (5.00 g, 28.8 mmol) in anhydrous DCM (100 mL) at 0 °C under N_2. The reaction mixture was allowed to warm slowly to rt, and stirring was continued for 3 h. The mixture was diluted with ice/water (300 mL), and the DCM layer was separated. The aqueous phase was extracted with EtOAc (3 × 100 mL). The aqueous layer was then acidified with conc. HCl and reextracted with EtOAc (3 × 100 mL). The combined organic phase was washed with brine (300 mL), dried, and evaporated. The residue was subjected to flash column chromatography. Elution with DCM afforded phenol **51** as a white solid (4.00 g 85%), mp 180−133 °C. R_f (DCM) 0.3. IR (ATR) ν_{max} cm^{-1}: 3200−3000 (OH), 1676 (C═O). ^1H NMR (400 MHz, $CDCl_3$) δ 12.28 (s, 1H, OH), 6.99 (d, J = 8.8 Hz, 1H, H3), 6.94 (d, J = 7.5 Hz, 1H, H6), 6.81 (d, J = 7.3 Hz, 1H, H7), 6.79 (d, J = 8.6 Hz, 1H, H4) 3.84 (s, 3H, OMe), 3.53 (s 3H, NMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 (C1), 153.5 (C8), 145.6 (C5), 133.1 (C3), 127.6 (C4a or C8a), 115.4 (C8a), 111.4 (C6 or C7), 111.3 (C6 or C7), 101.2 (C4), 56.2 (OMe), 36.0 (NMe). HRMS (APCI) m/z: calcd for $C_{11}H_{12}NO_3^+$ $[M + H]$ + 206.0812; found 206.0814.

*8-Hydroxy-5-methoxy-2-methyl-7-nitroisoquinolin-1(2H)-one (**52**).* A solution of 69% HNO_3 (0.19 mL, 2.9 mmol) in AcOH (3 mL) was added dropwise to a stirred solution of the phenol **51** (0.60 g, 2.9 mmol) in AcOH (3 mL) at such a rate as to maintain the temperature between 10 and 15 °C (50 min). Stirring was continued for 45 min, then water (10 mL) was added, and the precipitate was collected by vacuum filtration, washed with water, and air-dried to give **52** as a brown solid (0.67 g, 91%), mp 200−204 °C. R_f 0.3 (1:19 MeOH/DCM). IR (ATR) ν_{max} cm^{-1}: 1656 (C═O). ^1H NMR

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(500 MHz, DMSO-d_6) δ 14.55 (s, 1H, OH), 7.82 (d, J = 7.5 Hz, 1H, H3), 7.68 (s, 1H, H6), 6.89 (d, J = 7.4 Hz, 1H, H4), 3.92 (s, 3H, OMe), 3.60 (s, 3H, NMe). ^{13}C NMR (125 MHz, DMSO-d_6), 165.2 (C1), 151.2 (C8), 144.4 (C5), 137.9 (C3), 133.9, 130.6, 112.2, 108.5 (C6), 101.1 (C4), 56.4 (OMe), 36.6 (NMe). HRMS (APCI): calcd for $C_{11}H_{11}N_2O_5^+$ [M + H]$^+$ 251.0662; found 251.0661.

*7-Amino-8-hydroxy-5-methoxy-2-methylisoquinolin-1(2H)-one (**53**).* $SnCl_2{\cdot}2H_2O$ (3.00 g, 13.32 mmol) was added to a stirred solution of **52** (0.66 g, 2.7 mmol) in EtOH (20 mL), and the mixture was heated under reflux for 1.5 h. The reaction mixture was cooled at rt, then poured into ice (150 g), and the resulting suspension was made alkaline by an addition of sat. aq. $NaHCO_3$. The aqueous phase was extracted with DCM (3 × 25 mL), and the extract was dried and evaporated. The residue was diluted with 1 M HCl and washed with DCM. The aqueous layer was made alkaline by addition of $NaHCO_3$ and reextracted with DCM (3 × 25 mL). The extract was washed with brine (25 mL), dried, and evaporated to give aniline **53** as a yellow solid (0.48 g, 81%), mp 140−143 °C. R_f (1:19 EtOAc/hexanes) 0.2. IR (ATR) ν_{max} cm^{-1}: 3400−2800 (OH/NH), 1652 (C═O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.40 (s, 1H, OH), 7.04 (d, J = 7.5 Hz, 1H, H3), 6.79 (s, 1H, H6), 6.64 (d, J = 7.5 Hz, 1H, H4), 5.00 (br s, 2H, NH_2), 3.77 (s, 3H, OMe), 3.46 (s, 3H, NMe). ^{13}C NMR (500 MHz, DMSO-d_6) δ 164.8 (C═O), 146.1 (C5), 138.4 (C8), 134.3, 127.5 (C3), 115.8, 110.9, 103.5 (C4 or C6), 102.0 (C4 or C6), 55.9 (OMe), 35.7 (NMe). HRMS (APCI): calcd for $C_{11}H_{13}N_2O_3^+$ [M + H]$^+$ 221.0921; found 251.0924.

*8-Hydroxy-5-methoxy-7-methoxycarbonylamino-2-methylisoquinolin-1(2H)-one (**54**).* Methyl chloroformate (0.7 mL, 9 mmol) was added dropwise to a stirred solution of aniline **53** (1.98 g, 8.99 mmol) in anhydrous pyridine (30 mL) at 0 °C. After 0.5 h, the ice bath was removed and stirring was continued for 13 h. The reaction mixture was poured onto ice (∼200 mL), carefully acidified with conc. HCl, and extracted with EtOAc (4 × 50 mL). The extract was washed with water (2 × 50 mL) and brine (50 mL), dried, and evaporated to give the methyl carbamate **54** as a white solid (2.00 g, 80%), mp 132−133 °C. R_f (7:3 EtOAc/hexanes) 0.4. IR (ATR) ν_{max} cm^{-1}: 3200−2800 (NH/OH), 1709 (O═COMe), 1655 (O═C1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.84 (s, 1H, OH), 8.69 (s, 1H, NH), 7.64 (br s, 1H, H6), 7.38 (d, J = 7.5 Hz, 1H, H3), 6.76 (d, J = 7.5 Hz, 1H, H4), 3.82 (s, 3H, OMe), 3.67 (s, 3H, OMe), 3.52 (s, 3H, NMe). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8 (C1), 154.5 (CO_2), 145.0 (C5 or C8), 144.4 (C5 or C8), 131.9 (C3), 126.2, 123.3, 122.1, 111.1 (C6), 101.3 (C4), 56.1 (OMe), 51.9 (CO_2Me), 36.0 (NMe). HRMS (APCI): calcd for $C_{13}H_{15}N_2O_5^+$ [M + H]$^+$ 279.0975; found 279.0971.

*7-Acetylamino-2-methylisoquinoline-1,5,8-trione (**56**).* A solution of CAN (0.74 g, 1.4 mmol) in H_2O (5 mL) was added to a stirred solution of **46** (44 mg, 0.16 mmol) in MeCN (15 mL) at 0 °C. After 10 min, the reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave quinone **56** as a red-orange solid (30 mg, 77%), mp 230−233 °C. R_f (1:19 MeOH/DCM) 0.35. IR (ATR) ν_{max} cm^{-1}: 3257 (NH), 1682 (O═CNH), 1644 (O═C1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (s, 1H, NH), 8.37 (d, J = 6.6 Hz, 1H, H3), 7.54 (s, 1H, H6), 6.66 (d, J = 6.6 Hz, 1H, H4), 3.54 (s, 3H, NMe), 2.23 (s, 3H, $COCH_3$). ^{13}C NMR (150 MHz, DMSO-d_6) δ 184.8 (C8), 177.4 (C5), 171.4 (OCNH), 157.3 (C1), 148.7 (C3), 143.2 (C4a or C7), 142.1 (C4a or C7), 115.4 (C8a), 112.8 (C6), 99.6 (C4), 38.2 (NMe), 24.7 (CH_3). HRMS (ESI): calcd for $C_{12}H_{11}N_2O_4^+$ [M + H]$^+$ 247.0714; found 247.0738.

*7-Methoxycarbonylamino-2-methylisoquinoline-1,5,8-trione (**57**).* A solution of CAN (0.16 g, 0.29 mmol) in H_2O (1 mL) was added to a stirred solution of **47** (28 mg, 95 μmol) in MeCN (6 mL) at 0 °C. After 10 min, the reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated to give quinone **57** as a red-orange solid (24 mg, 95%), mp 189−193 °C. R_f (1:19 MeOH/DCM) 0.1. IR (ATR) ν_{max} cm^{-1}: 3356 (NH), 1742 (O═C), 1690 (O═C1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.18 (s, 1H, NH), 8.38 (d, J = 6.6 Hz, 1H, H3), 7.2 (s, 1H, H6), 6.68 (d, J = 6.6 Hz, 1H, H4), 3.74 (s, 3H, OMe), 3.54 (s, 3H, NMe). ^{13}C NMR (150 MHz, DMSO-d_6) δ 184.0 (C8), 176.6 (C5), 157.3 (C1), 153.2 (CO_2), 148.8 (C3), 143.4 (C4a or C7), 142.9 (C4a or C7), 115.4 (C8a), 111.5 (C6), 99.7 (C4), 52.9 (OMe), 38.2 (NMe). HRMS (APCI): calcd for $C_{12}H_{11}N_2O_5^+$ [M + H]$^+$ 263.0663; found 263.0675.

*7-t-Butoxycarbonylamino-2-methylisoquinoline-1,5,8-trione (**58**).* A solution of CAN (4.8 g, 8.8 mmol) in H_2O (10 mL) was added to a stirred solution of **48** (0.93 g, 2.77 mmol) in MeCN (30 mL) at 0 °C. After 10 min, the reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 20 mL). The extract was dried and evaporated to give **58** as a red-orange solid (0.59 g, 70%), mp 268−271 °C. R_f (EtOAc) 0.25. IR (ATR) ν_{max} cm^{-1}: 3380 (NH), 1746 (O═CO), 1621 (O═C1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (s, 1H, NH), 8.37 (d, J = 6.6 Hz, 1H, H3), 7.13 (s, 1H, H6), 6.67 (d, J = 6.6 Hz, 1H, H4), 3.54 (s, 3H, NMe), 1.48 (s, 9H, tBu). ^{13}C NMR (150 MHz, DMSO-d_6) δ 183.8 (C5), 176.6 (C8), 157.3 (C1), 151.4 (CO_2), 148.9 (C3), 143.5 (C4a or C7), 142.7 (C4a or C7), 115.3 (C8a), 111.0 (C6), 99.8 (C4), 82.0 (t-Bu−O), 38.2 (NMe), 27.7 (3 × CH_3). HRMS (APCI): calcd for $C_{15}H_{17}N_2O_5^+$ [M + H]$^+$ 305.1132; found 305.1127.

*5,8-Dimethoxy-7-methoxycarbonyl(methyl)amino-2-methyl-4,6-dinitroisoquinolin-1(2H)-one (**61**).* A solution of CAN (0.42 g, 0.77 mmol) in H_2O (1 mL) was added to a stirred solution of **49** (79 mg, 0.26 mmol) in MeCN (3 mL) at 0 °C. After 45 min, the reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated to give a residue that was purified by flash column chromatography. Elution with 9:1 EtOAc/hexanes afforded **61** as a yellow solid (67 mg, 63%), mp 160−163 °C. R_f (9:1 EtOAc/hexanes) 0.3. IR (ATR) ν_{max} cm^{-1}: 1687 (C═O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (s, 1H, H3), 3.98 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.75 (s, 3H, OMe), 3.42 (s, 3H, NMe), 3.29 (s, 3H, NMe). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.8, 160.6 (C1), 157.8, 157.6, 155.8, 151.4. 147.1, 124.3, 118.9, 118.4, 62.3 (OMe), 57.1 (OMe), 53.6 CO_2Me), 37.5 (NMe), 27.6 (NMe).

*5,8-Dimethoxy-2-methyl-7-methylaminoisoquinolin-1(2H)-one (**62**).* A solution of CAN (0.28 g, 0.52 mmol) in H_2O (1 mL) was added to a stirred solution of carbamate **50** (60 mg, 0.17 mmol) in acetonitrile (2 mL) at 0 °C. After 10 min, the reaction mixture was diluted with H_2O (3 mL) and extracted with EtOAc (3 × 10 mL). The organic extract was dried and evaporated to give quinone **62** as a pale-yellow solid (35 mg, 78%). R_f (2:23 MeOH/DCM) 0.3. IR (ATR) ν_{max} cm^{-1}: 3400 (NHMe), 1670 (C═O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.77 (d, J = 7.4, Hz, 1H, H3), 6.69 (dd, J = 7.4,

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0.5 Hz, 1H, H4), 6.52 (br s, 1H, H6), 4.79 (v. br s, 1H, NH), 3.91 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.51 (s, 3H, 2-NMe), 2.93 (s, 3H, 7-NMe). HRMS (APCI): calcd for $C_{13}H_{17}N_2O_3^+$ $[M + H]^+$ 249.1234; found 235.1236.

*Ethyl (7-Methoxycarbonylamino-2-methylisoquinoline-1,5,8-trion-6-yl)-2-(2-nitrophenyl)acetate (**64**).* A mixture of K_2CO_3 (26 mg, 0.19 mmol), **57** (16 mg, 61 μmol), and ethyl *o*-nitrophenylacetate (**2**) (37 mg, 0.18 mmol) in dry DMF (15 mL) was stirred under Ar at 40 °C for 24 h, then cooled, diluted with 1 M HCl (20 mL), and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave **64** as an orange oil (20 mg, 71%). R_f (1:19 MeOH/DCM) 0.1. IR (ATR) ν_{max} cm^{-1}: 1736 (O═C), 1691 (O═C1). ^1H NMR (600 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 1H, H3″), 7.87 (d, J = 7.2 Hz, 1H, H3′), 7.81 (s, 1H, NH), 7.51 (dd [app. t], $J_1 = J_2$ = 8.4 Hz, 1H, H4″ or H5″), 7.42 (dd [app. t], $J_1 = J_2$ = 8.4 Hz, 1H, H4″ or H5″), 7.28 (d, J = 8.4 Hz, 1H, H6″), 6.84 (d, J = 7.2 Hz, 1H, H4′), 6.00 (s, 1H, H2), 4.21 (m, 1H, OCH_2-a), 4.14 (m, 1H, OCH_2-b), 3.68 (OMe or NMe), 3.66 (OMe or NMe), 1.16 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.8 (C5′), 178.0 (C8′), 169.7 (C1), 157.8 (C1′), 153.0 (NCO_2), 149.9 (C2″), 146.5 (C3′), 144.2 (C4a′), 141.7 (C6″), 132.6 (C4″ or C5″), 131.7 (C1″), 130.9 (C4″ or C5″), 128.2 (C3″ or C6″), 127.0 (C7′), 124.9 (C3″ or C6″), 116.8 (C8a′), 101.5 (C4′), 62.0 (OCH_2), 53.9 (OMe), 47.0 (C2), 39.2 (NMe), 14.0 ($OCH_2\mathbf{CH_3}$). HRMS (APCI): calcd for $C_{22}H_{20}N_3O_9^+$ $[M + H]^+$ 470.1195; found 470.1219.

*Ethyl (7-t-Butoxycarbonylamino-2-methylisoquinoline-1,5,8-trion-6-yl)-2-(2-nitrophenyl)acetate (**65**).* A solution of **58** (0.56 g, 1.8 mmol) in DMF (30 mL) was added slowly to a stirred suspension of K_2CO_3 (1.4 g, 10 mmol), ethyl *o*-nitrophenylacetate (**2**) (1.3 g, 6.2 mmol), and 18-crown-6 (52 mg, 0.19 mmol) in dry DMF (50 mL) at 45 °C under Ar. After 24 h, the reaction mixture was cooled, diluted with 1 M HCl (50 mL), and extracted with EtOAc (3 × 25 mL). The extract was dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:19 MeOH/DCM gave **65** as an orange oil (0.43 g, 46%). R_f (1:19 MeOH/DCM) 0.2. IR (ATR) ν_{max} cm^{-1}: 1732 (O═CO), 1689 (O═C1). ^1H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, J = 7.8 Hz, 1H, H3″), 7.89 (d, J = 6.6 Hz, 1H, H3′), 7.66 (s, 1H, NH), 7.46 (dd [app. t], $J_1 = J_2$ = 7.8 Hz, 1H, H5″), 7.39 (dd [app. t], $J_1 = J_2$ = 7.8 Hz, 1H, H4″), 7.22 (d, J = 7.8 Hz, 1H, H6″), 6.80 (d, J = 6.6 Hz, 1H, H4′), 6.01 (s, 1H, H2), 4.18 (m, 1H, OCH_2-a), 4.10 (m, 1H, OCH_2-b), 3.65 (s, 3H, NMe), 1.37 (s, 9H, *t*Bu), 1.14 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.7 (C5′), 178.3 (C8′), 169.9 (C1), 157.8 (C1′), 151.2 (HNC═O), 149.7 (C2″), 146.7 (C3′), 144.2 (C4a′), 142.3 (C6′), 132.7 (C4″ or C5″), 131.8 (C1″), 130.7 (C4″ or C5″), 128.0 (C3″ or C6″), 126.4 (C7′), 124.8 (C3″ or C6″), 116.7 (C8a′), 101.5 (C4′), 83.1 (*t*-Bu−O), 61.9 (OCH_2), 47.1 (C2), 39.1 (NMe), 27.9 (*t*Bu), 13.9 ($OCH_2\mathbf{CH_3}$). HRMS (ESI^-): calcd for $C_{25}H_{24}N_3O_9^-$ $[M-H]^-$ 510.1518; found 510.1518.

*Ethyl 2-(7-Amino-8-hydroxy-2-methyl-1,5-dioxo-1,2-dihydroisoquinolin-6(5H)-ylidene)-2-(2-nitrophenyl)acetate (**66**).* Ethanolic HCl was prepared by adding AcCl (5 mL, 0.3 mol) to EtOH (10 mL) at 0 °C. The resulting solution was then added dropwise to a solution of **65** (0.15 g, 0.29 mmol) in EtOH (20 mL). After 20 h, the solution was heated under reflux for 30 min before being cooled, diluted with H_2O (30 mL), and extracted with EtOAc (3 × 10 mL). The extract was dried and evaporated then triturated with hot 1:9 EtOAc/hexanes, leaving what has been tentatively assigned as **66** as a purple solid (62 mg, 51%), mp 270−273 °C. R_f (1:19 MeOH/DCM) 0.15. IR (ATR) ν_{max} cm^{-1}: 3300−2800 (OH), 1658 (O═C1). ^1H NMR (600 MHz, DMSO-d_6) δ 8.25 (d, J = 6.6 Hz, 1H, H3′), 8.03 (d, J = 8.4 Hz, 1H, H3″ or H6″), 7.71 (dd [app. t], $J_1 = J_2$ = 8.4 Hz, 1H, H4″ or H5), 7.62−7.55 (m, 2H, ArH), 6.63 (d, J = 6.6 Hz, 1H, H4″), 4.20 (q, J = 7.2 Hz, 2H, OCH_2), 3.52 (s, 3H, NMe), 1.16 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$). ^{13}C NMR (150 MHz, DMSO-d_6) δ 179.2 (C5′), 173.0 (C1), 157.7 (C1′), 148.5 (C3′), 146.7, 145.2, 133.4 (ArH), 132.6 (ArH), 128.2, 126.0, 124.2 (ArH), 119.9, 116.3, 101.1 (ArH), 99.9 (ArH), 68.7 (OCH_2), 37.9 (NMe), 14.4 ($OCH_2\mathbf{CH_3}$).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02117.

^1H and ^{13}C spectra annotated with skeleton-numbered structures; general crystallographic methods and tabulated data for compounds **36** and **37** (PDF)

Individual CIF files and CCDC deposit numbers for these crystal structures (CIF, CIF)

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Notes

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■ ADDITIONAL NOTE

[a]As noted in the Results and Discussion section, when this reaction was repeated several years later, the yields of **36**:**37** were inverted to 13:76%.

■ REFERENCES

(1) Buccini, M.; Jeow, S. Y.; Byrne, L.; Skelton, B. W.; Nguyen, T. M.; Chai, C. L. L.; Piggott, M. J. Bisannulation of 2,3-dichloro-1,4-naphthoquinone with *o*-nitrophenylacetic acid derivatives: a succinct synthesis of the ABCD ring system of alpkinidine. *Eur. J. Org. Chem.* **2013**, 3232−3240.

(2) Buccini, M.; Tham, L.; Dhoro, F.; Skelton, B. W.; Williams, C. M.; Piggott, M. J. Towards the Total Synthesis of Alpkinidine: Synthesis of Haloquinone CE Ring-System Synthons and Attempted Nucleophilic Bisannulation. *ACS Omega* **2022**, DOI: 10.1021/acsomega.2c02116.

(3) Thale, Z.; Johnson, T.; Tenney, K.; Wenzel, P. J.; Lobkovsky, E.; Clardy, J.; Media, J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. Structures and cytotoxic properties of sponge-derived bisannulated acridines. *J. Org. Chem.* **2002**, *67*, 9384−9391.

(4) Kitahara, Y.; Mizuno, T.; Kubo, A. Synthetic studies of benzo[*b*]pyrrolo[4,3,2-*de*][1,10]phenanthroline. *Tetrahedron* **2004**, *60*, 4283−4288.

(5) Komori, T.; Yokoshima, S.; Fukuyama, T. Synthetic studies on plakinidines. *Synlett* **2015**, *26*, 1537−1540.

(6) Volvoikar, P. S.; Tilve, S. G.; Zubkov, F. I. A concise approach for the synthesis of the ABCD ring system of alpkinidine. *ChemistrySelect* **2019**, *4*, 7187−7189.

(7) Rho, H. S.; Baek, H. S.; Ahn, S. M.; Yoo, J. W.; Kim, D. H.; Kim, H. G. Studies on depigmenting activities of dihydroxyl benzamide derivatives containing adamantane moiety. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1532−1533.

(8) Prey, V. Cleavage of phenol ethers with pyridine hydrochloride. *Ber. Dtsch. Chem. Ges. B* **1941**, *74*, 1219−1225.

(9) Hofslokken, N. U.; Skattebol, L. Convenient method for the *ortho*-formylation of phenols. *Acta Chem. Scand.* **1999**, *53*, 258−262.

(10) Evans, R. M.; Downes, M.; Baiga, T. J.; Noel, J. P.; Embler, E. K.; Fan, W.; Keana, J. F. W.; Bock, M. G.; Kluge, A. F.; Patane, M. A. Preparation of phenoxy hexanoic acid compounds as PPARγ agonists for therapy. United States patent, US20160023991 A1 2016-01-28.

(11) Baker, R.; Castro, J. L. Total synthesis of (+)-macbecin I. *J. Chem. Soc., Perkin Trans. 1* **1990**, 47−65.

(12) Godard, A.; Fourquez, J. M.; Tamion, R.; Marsais, F.; Quéguiner, G. New synthesis of the streptonigrin quinoline-5,8-quinone moiety and aromatic cross-coupling. *Synlett* **1994**, 235−236.

(13) Chawla, H. M.; Mittal, R. S. Cerium (IV) induced one pot synthesis of 2,4-dihydroxy-3-nitroacetophenone - a model for biomimetic aromatic hydroxylations. *Curr. Sci.* **1983**, *52*, 1189−1190.

(14) Chakrabarty, M.; Batabyal, A. A new method of nitration of carbazoles using ceric ammonium nitrate (CAN). *Synth. Commun.* **1994**, *24*, 1−10.

(15) Ganguly, N.; Sukai, A. K.; De, S. Cerium(IV) ammonium nitrate mediated nitration of coumarins. *Synth. Commun.* **2001**, *31*, 301−309.

(16) Yang, X.; Xi, C.; Jiang, Y. Regioselective nitration of *N*,*N*-dialkylanilines using cerium(IV) ammonium nitrate in acetonitrile. *Tetrahedron Lett.* **2005**, *46*, 8781−8783.

(17) Tohma, H.; Morioka, H.; Harayama, Y.; Hashizume, M.; Kita, Y. Novel and efficient synthesis of *p*-quinones in water via oxidative demethylation of phenol ethers using hypervalent iodine(III) reagents. *Tetrahedron Lett.* **2001**, *42*, 6899−6902.

(18) Hwu, J. R.; Jain, M. L.; Tsay, S.-C.; Hakimelahi, G. H. Ceric ammonium nitrate in the deprotection of *tert*-butoxycarbonyl group. *Tetrahedron Lett.* **1996**, *37*, 2035−2038.

(19) Evans, E. F.; Lewis, N. J.; Kapfer, I.; Macdonald, G.; Taylor, R. J. K. *N*-*tert*-Butoxycarbonyl (BOC) deprotection using boron trifluoride etherate. *Synth. Commun.* **1997**, *27*, 1819−1825.

(20) Houghten, R. A.; Beckman, A.; Ostresh, J. M. Use of 10% sulfuric acid/dioxane for removal of *N*-α-tertiary-butyloxycarbonyl group during solid phase peptide synthesis. *Int. J. Pept. Protein Res.* **1986**, *27*, 653−658.

(21) De Guzman, F. S.; Carte, B.; Troupe, N.; Faulkner, D. J.; Harper, M. K.; Concepcion, G. P.; Mangalindan, G. C.; Matsumoto, S. S.; Barrows, L. R.; Ireland, C. M. Neoamphimedine: a new pyridoacridine topoisomerase II inhibitor which catenates DNA. *J. Org. Chem.* **1999**, *64*, 1400−1402.

(22) Tasdemir, D.; Marshall, K. M.; Mangalindan, G. C.; Concepcion, G. P.; Barrows, L. R.; Harper, M. K.; Ireland, C. M. Deoxyamphimedine, a new pyridoacridine alkaloid from two tropical *Xestospongia* sponges. *J. Org. Chem.* **2001**, *66*, 3246−3248.

(23) Marshall, K. M.; Matsumoto, S. S.; Holden, J. A.; Concepcion, G. P.; Tasdemir, D.; Ireland, C. M.; Barrows, L. R. The anti-neoplastic and novel topoisomerase II-mediated cytotoxicity of neoamphimedine, a marine pyridoacridine. *Biochem. Pharmacol.* **2003**, *66*, 447−458.

(24) Dhoro, F.; Parkin-Gibbs, J.; McIldowie, M.; Skelton, B. W.; Piggott, M. J. Confirmation of the revised structure of samoquasine A and a proposed structural revision of cherimoline. *J. Nat. Prod.* **2018**, *81*, 1658−1665.

(25) Chatterjea, J. N.; Jha, H. C.; Banerjee, B. K. Isocarbostyrils. I. Conversion of 8-alkoxyisocarbostyril-3-carboxylic acids into the related hydroxy esters by pseudo rearrangement. *J. Indian Chem. Soc.* **1966**, *43*, 633−647.

(26) McGarrigle, E. M.; Murphy, D. M.; Gilheany, D. G. Ligand tuning in the chromium-salen-mediated asymmetric epoxidation of alkenes. *Tetrahedron: Asymmetry* **2004**, *15*, 1343−1354.

(27) Rubenstein, L. Substitution in derivatives of quinol ethers. *J. Chem. Soc., Trans.* **1925**, *127*, 1998−2004.

(28) Kashin, D.; Meyer, A.; Wittenberg, R.; Schoening, K.-U.; Kamlage, S.; Kirschning, A. A practical synthesis of the ansa chain of benzenic ansamycin antibiotics: total synthesis of an ansatrienol derivative. *Synthesis* **2007**, 304−319.

(29) Klemenc, A. Nitrogentisic acid. *Monatsh. Chem.* **1913**, *33*, 1243−1254.

(30) Yan, Y.; Qin, B.; Ren, C.; Chen, X.; Yip, Y. K.; Ye, R.; Zhang, D.; Su, H.; Zeng, H. Synthesis, structural investigations, hydrogen-deuterium exchange studies, and molecular modeling of conformationally stabilized aromatic oligoamides. *J. Am. Chem. Soc.* **2010**, *132*, 5869−5879.

https://doi.org/10.1021/acsomega.2c02117
ACS Omega 2022, 7, 19093−19105