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Levoglucosenone and Its Pseudoenantiomer *iso*-Levoglucosenone as Scaffolds for Drug Discovery and Development

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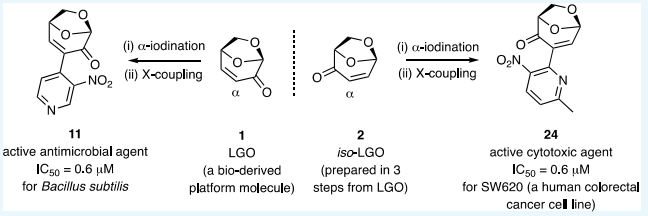
ABSTRACT: The bioderived platform molecule levoglucosenone (LGO, **1**) and its readily prepared pseudoenantiomer (*iso*-LGO, **2**) have each been subjected to α-iodination reactions with the product halides then being engaged in palladium-catalyzed Ullmann cross-coupling reactions with various bromonitropyridines. The corresponding α-pyridinylated derivatives such as **11** and **24**, respectively, are produced as a result. Biological screening of such products reveals that certain of them display potent and selective antimicrobial and/or cytotoxic properties. In contrast, the azaindoles obtained by reductive cyclization of compounds such as **11** and **12** are essentially inactive in these respects. Preliminary mode-of-action studies are reported.



INTRODUCTION

The azaindoles represent privileged structures in medicinal chemistry and many derivatives thereof have been explored as drug development candidates for the treatment of a range of diseases.[1] Of particular note has been the exploitation of such compounds as kinase inhibitors,[2] as antiviral agents,[3] as antitumor agents,[2,4] as cannabinoid (CB) receptor agonists,[5] and as potential treatments for diabetes.[6] These same heterocycles are also encountered, albeit rather infrequently, in biologically active natural products such as the variolins,[7] the marinoquinolines,[8] and the guitarrins.[9]

Recently, we reported the use of a palladium-catalyzed Ullmann cross-coupling/reductive cyclization protocol[10] for the construction of 2,3-annulated-azaindoles from halonitropyridines and α-iodinated cyclohex-2-en-1-ones.[11] Simultaneously, we have been exploring the chemistry of the homochiral and α,β-unsaturated ketone levoglucosenone (LGO, **1**) (Figure 1),[12] a compound that is now produced commercially in tonne quantities through the high-temperature pyrolysis of acid-treated cellulose.[13] In addition, the pseudoenantiomeric compound *iso*-levoglucosenone (*iso*-LGO, **2**) can be obtained in just three steps and on a multigram scale[12c] from congener **1**.[14] While compounds **1** and **2** are now abundant materials, they remain little explored as scaffolds for drug discovery.[15] Accordingly, we report herein on the deployment of their α-iodinated derivatives as reaction partners in the assembly, via the abovementioned, two-step protocol, of a suite of novel and homochiral azaindoles together with the evaluation of these and their precursors in a range of antimicrobial and anticancer screens. As revealed below, while the targeted heterocycles show little if any biological effects, certain of their nitropyridine-containing precursors are notably active.

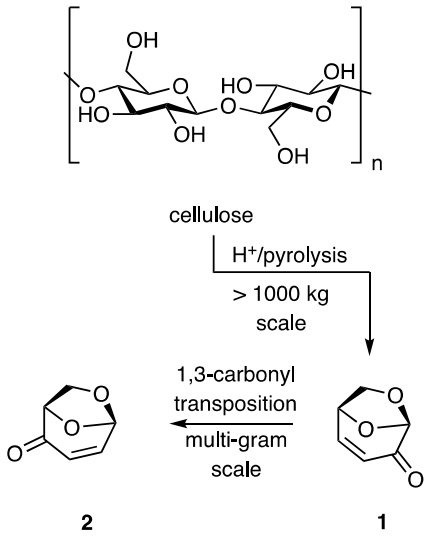


Figure 1. Structures of LGO (**1**) and *iso*-LGO (**2**) and their scalable modes of production.

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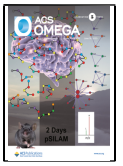

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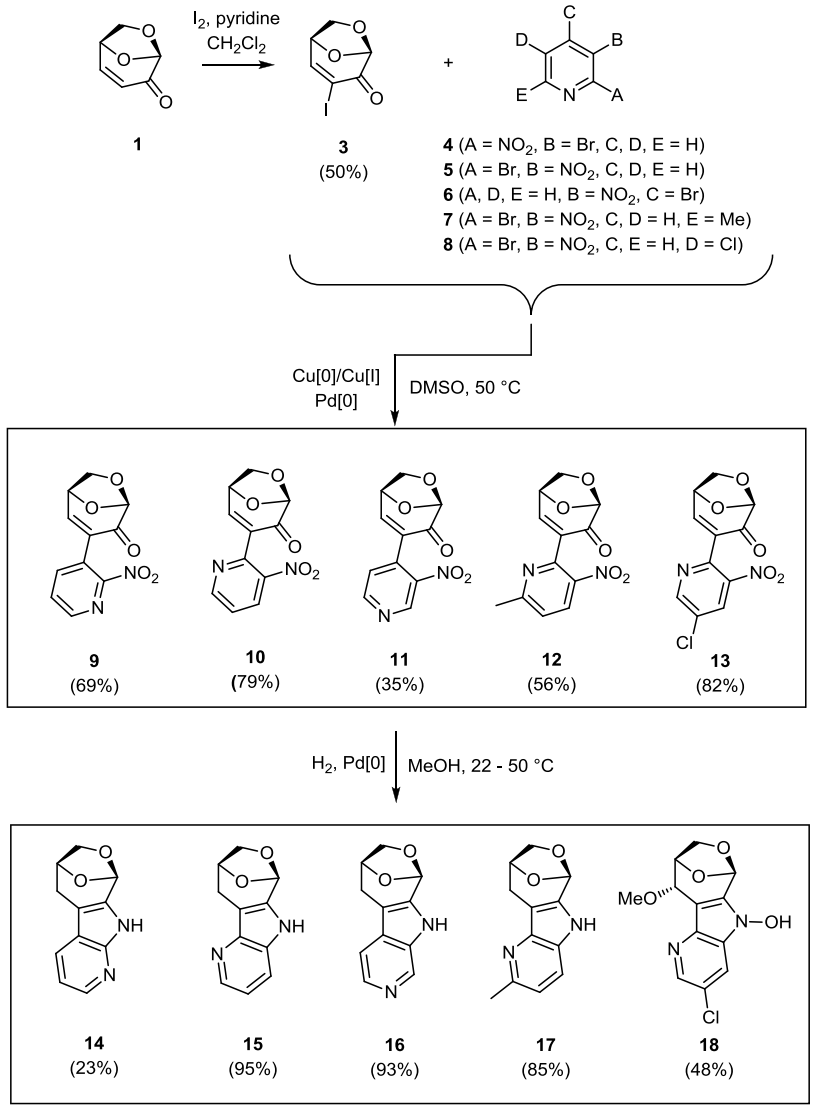
Scheme 1. Synthetic Sequence Leading from LGO (1) to Test Compounds 9−18



RESULTS AND DISCUSSION

The simple reaction sequence allowing for the elaboration of LGO into a series of α-nitropyridinylated derivatives and thence, through reductive cyclization of these, into the corresponding azaindoles is shown in Scheme 1. Thus, α-iodination of LGO (**1**) under conditions defined by Isobe and co-workers gave derivative **3**[16] (50%) that was then engaged in palladium-catalyzed cross-couplings with the bromonitropyridines **4**−**8**[11] and thereby affording the products **9**−**13**, respectively, in yields ranging from 35 to 82%. The structures of two of these (**10** and **13**) were confirmed by single-crystal X-ray analysis (see the Supporting Information (SI) for details). In an operationally simple process, when methanolic solutions of each of the compounds **9**−**13** were exposed to hydrogen in the presence of palladium on carbon at 22−50 °C, reductive cyclization reactions took place and, in one case (**13**), solvent incorporation also occurred. As a result the azaindole derivatives **14**−**18**, respectively, were obtained. The lowest yielding process was the one leading to the 7-azaindole **14**, while incomplete reductive cyclization was observed in the case of substrate **13**, and such that the *N*-hydroxyazaindole **18** was obtained. Once again, the structures of certain of these compounds (**14**, **16**, and **17**) were confirmed by single-crystal X-ray analysis (see the SI for details).

The *iso*-LGO-containing azaindoles and their precursors were prepared by the analogous means shown in Scheme 2. Thus, *iso*-LGO (**2**) was α-iodinated under Isobe's conditions to give the previously unreported halide **19** (66%), which was cross-coupled with the bromonitropyridines **4**−**8** and **20**, thereby affording the corresponding products **21**−**26** (46−84%) with the structures of the second and third of these being confirmed by X-ray analyses (see SI). The outcomes of the reductive cyclizations of compounds **21**−**26** were quite varied with the reactions of the first four of these substrates under the same conditions as used earlier, leading to identifiable products, *viz*. **27**−**30** (48−89%) although single-crystal X-ray analyses of the third and fourth of these revealed (see SI) that the former (*viz*. **29**) was a 1-hydroxy-6-azaindole and not the corresponding, fully reduced system. For reasons that remain unclear, attempts to reduce substrates **25** and **26** under the same or related conditions failed to provide the anticipated azaindoles. In the case of the former compound, complex product mixtures were observed, while in the case of the latter this proved inert to all of the reaction conditions examined.

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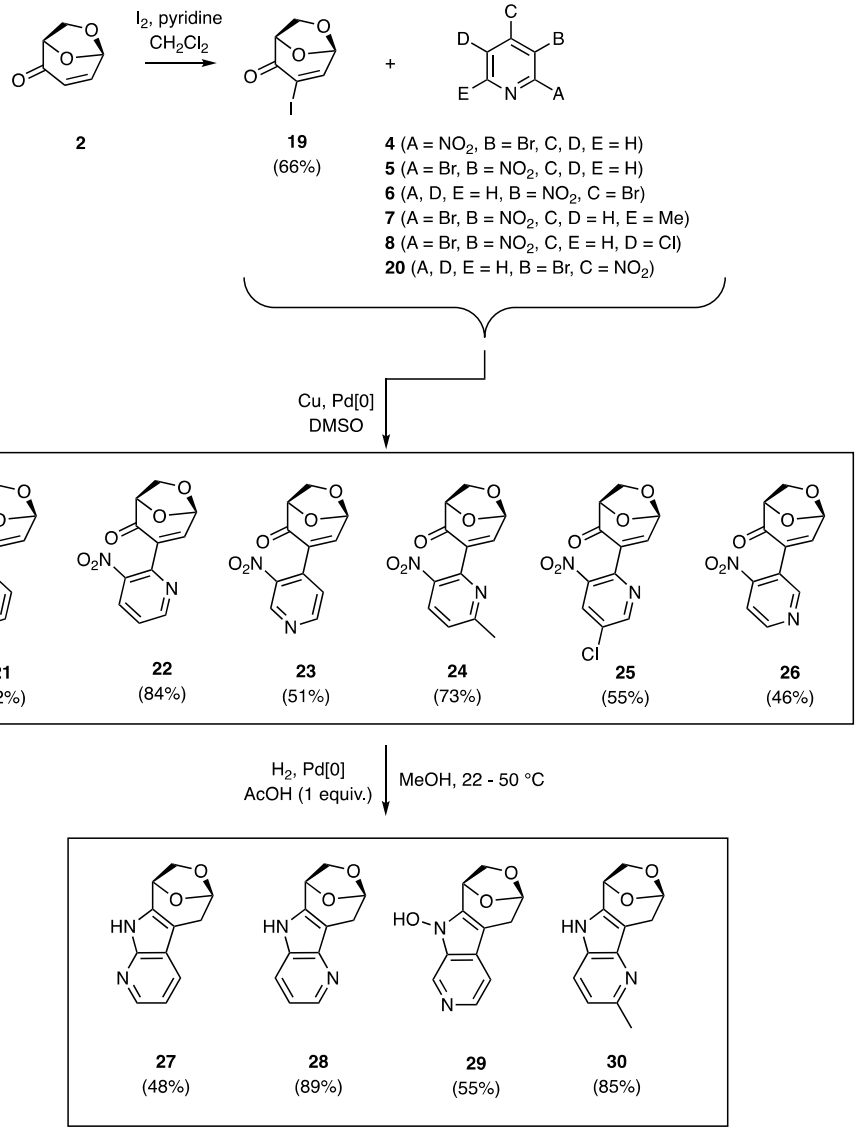
Scheme 2. Synthetic Sequence Leading from *iso*-LGO (2) to Test Compounds 21−30



In our earlier work, we disclosed[11b] methods for the reductive cyclization of various α-arylated, α,β-unsaturated carbonyl-containing systems using iron or $TiCl_3$ under aqueous acidic conditions and thereby affording heterocyclic systems regioisomeric with those obtained using the H_2/Pd on C combination. On applying the first of these alternative conditions to substrate **9** and using aqueous acetic acid, ethanol, or aqueous hydrochloric acid as the reaction medium, compounds **31**, **32**, and **33**[11b] (Figure 2), respectively, were obtained. Once again, the structures of the last two of these were confirmed by single-crystal X-ray analyses (see SI). The precise modes of formation of these distinct products from the common starting material **9** are the subject of ongoing investigations.

All of the LGO-derived cross-coupling products **9**−**13**, the derived azaindoles **14**−**18** and **31**−**33**, as well as the corresponding collection of *iso*-LGO-derived systems, *viz.* compounds **21**−**30**, were subject to biological evaluation against a panel of six antibiotic-sensitive and two antibiotic-resistant Gram-positive and -negative bacteria together with the fungus *Candida albicans* ATCC90028. In essentially every instance (see SI), the azaindoles proved inactive while, in

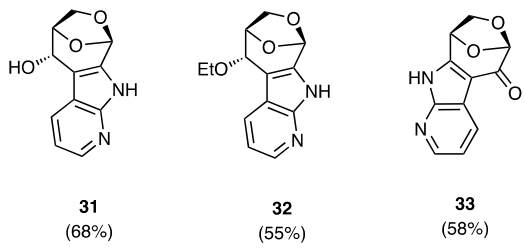


Figure 2. Structures of the products **31**−**33** arising from the reductive cyclization of cross-coupling product **9** using iron in various acidic media.

marked contrast, certain of the precursor α-arylated LGO and *iso*-LGO derivatives proved to have significant antibacterial effects (Table 1). Notably, the LGO-derived compound **11** showed an IC_{50} value of 0.6 μM against *Bacillus subtilis* (ATCC6633).

Compounds **9**−**18** and **21**−**33** were also evaluated as cytotoxic agents against the HepG2, NCIH-460, and SW620 cell lines (which are human hepatocellular, lung, and colorectal cancer cell lines, respectively). Again, the activity of the

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Table 1. Outcomes of the Antibacterial Screening of Compounds 11,13, 21, 22, and 24−26[a]

		Staphylococcus aureus (ATCC25923)		methicillin-resistant *Staphylococcus aureus* (MRSA)		*Bacillus subtilis* (ATCC6633)		*Streptococcus pyogenes* (D3840)	
entry	compound	MIC (μM)	IC_{50} (μM)	MIC (μM)	IC_{50} (μM)	MIC (μM)	IC_{50} (μM)	MIC (μM)	IC_{50} (μM)
1	**11**	30	8.1	30	10.0	3.3	0.6	10	5.5
2	**13**	30	6.0	nt[b]	nt	30	13.5	30	10.0
3	**21**	30	10.7	nt	nt	30	10.4	30	10.3
4	**22**	30	6.8	nt	nt	30	6.8	30	15.9
5	**24**	30	14.8	nt	nt	30	11.5	30	10.0
6	**25**	30	27.8	nt	nt	30	10.0	30	10.0
7	**26**	30	20.0	30	12.3	10	2.9	30	10.7

[a]See the SI for further details. [b]nt, not tested.

azaindoles in these assays were of little note (see SI) but the same subset of α-nitropyridinylated LGO and *iso*-LGO precursors displayed significant effects (Table 2). Specifically,

Table 2. Outcomes of the Cytotoxicity Screening of Compounds 11,13, 21, 22, and 24−26[a]

entry	compound	HepG2 IC_{50} (μM)	SW620 IC_{50} (μM)	NCIH-460 IC_{50} (μM)
1	**11**	2.7	0.8	8.0
2	**13**	5.6	5.6	5.1
3	**21**	6.5	8.0	1.0
4	**22**	7.0	3.2	5.1
5	**24**	8.0	0.6	7.0
6	**25**	14.6	2.1	12.0
7	**26**	5.0	1.0	2.9

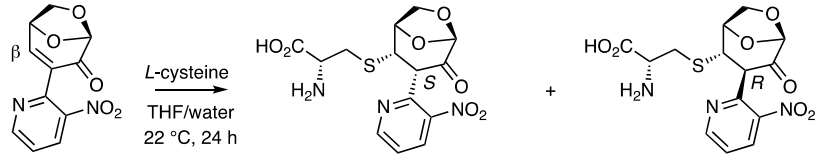
[a]See SI for further details.

compound **11** and the *iso*-LGO-derived system **24** display IC_{50} values of 0.8 and 0.6 μM, respectively, against the SW620 cell line. Compound **21** displays (entry 3, Table 2) similarly notable effects against the NCIH-460 cell line. As such, these compounds appear to possess cytotoxic properties that compare particularly favorably to other LGO-derived systems.[15]

The origins of the abovementioned and notable antimicrobial and/or cytotoxic properties of compounds **11**,**13**, **21**, **22**, and **24**−**26** are most likely connected with their capacities to act as Michael acceptors[15] and thereby engage in covalent binding with nucleophilic amino acid residues at the target receptor(s).[17] In support of such notions, we have observed that when equimolar mixtures of compounds **9**, **11**, or **21** and L-cysteine (or the corresponding methyl ester) are subject to electrospray ionization (ESI) mass spectrometric analysis (in either positive or negative mode), then prominent molecular-associated ions arising from their mutual conjugation are observed in each instance (this was not the case with the associated azaindoles). Furthermore, a solution-phase reaction of congener **10** (Scheme 3) with a half-molar equivalent[18] of L-cysteine in methanol/water under ambient conditions for 48 h afforded, after chromatographic purification, the adducts **34** (18%) and **35** (38%). Spectroscopic analysis of each of these clearly suggested that they are diastereoisomers arising from conjugate addition of the sulfhydryl group of the amino acid to the less-congested α-face of the substrate and therefore differ in the configuration (*R* vs *S*) of the nitropyridyl residue at the α-position of the LGO framework. The illustrated configurations of these diastereoisomers are tentatively assigned on the basis that the resonances due to the diastereotopic methylene protons of the cysteine side chain in the *cis*-isomer **34** are much more strongly differentiated than their counterparts in *trans*-isomer **35** because of the greater impact of the proximal α-nitropyridinyl residue in the former case ($\Delta\delta_H$ = 0.35 vs < 0.01). The latter conjugate, *viz*. compound **35** (which predominates), is presumed to be the thermodynamically more stable one[15d] (the fragile nature of compounds **34** and **35** prevented the conduct of relevant equilibration studies so as to determine if this is indeed the case).

These conjugation experiments clearly indicate that the α-pyridinylated LGO and *iso*-LGO systems reported here are effective Michael acceptors. The predominance of the later systems among the active compounds (see Tables 1 and 2) could be taken as suggesting that these are more disposed toward such addition reactions than their LGO-based counterparts (and perhaps because the inductive effects of the internal and γ-related acetal enhancing the electrophilic character of the adjacent β-carbon). Regardless of such details, the seeming capacity to engage both frameworks in conjugation reactions suggests that peptide-incorporated derivatives (*viz*. peptide−drug conjugates or PDCs)[19] could serve as targeted prodrug forms of the active compounds and thus allow for the assembly of highly selective antibacterial and/or antitumor compounds. Of course, such adducts or related (simpler) ones might simply serve as a means for stabilizing otherwise highly reactive Michael acceptors and

Scheme 3. Reaction of the α-Arylated and LGO-Derived Enone 10 with L-Cysteine Leading to the Isolable Adducts 34 and 35



10 **34** (18%) **35** (38%)

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thereby enhancing bioavailability and improving therapeutic potential.[20]

CONCLUSIONS

The results detailed above clearly demonstrate that the bioderived platform molecule LGO (**1**) and its ready accessible pseudoenantiomer *iso*-LGO (**2**) represent new and useful molecular scaffolds for drug development. As such they warrant further study in this regard, not least because of the significant array of derivatives likely to be available through manipulation of the associated α,β-unsaturated ketone residues. Of course, the internal acetal units within these compounds also provide capacities for their manipulation in distinct ways and so offer further potential for diversifying these frameworks.

EXPERIMENTAL SECTION

Chemical Synthesis Studies. *General Experimental Protocols.* Unless otherwise specified, proton (^{1}H) and proton-decoupled carbon [^{13}C{^{1}H}] NMR spectra were recorded at room temperature in base-filtered $CDCl_3$ on a Bruker spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei. For ^{1}H NMR spectra, signals arising from the residual protio-forms of the solvent were used as the internal standards. ^{1}H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or combinations of the above. The signal due to residual $CHCl_3$ appearing at δ_H 7.26 and the central resonance of the $CDCl_3$ "triplet" appearing at δ_C 77.16 were used to reference ^{1}H and ^{13}C{^{1}H} NMR spectra, respectively. Attenuated total reflectance (ATR) IR spectra were recorded on a Bruker Alpha-P instrument with samples being prepared by allowing a $CDCl_3$ or CH2Cl$_2$ solution of these to evaporate on the sampling plate before the spectrum was acquired. In some instances, solids were applied directly to the sampling plate. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at the sodium-D line (λ = 589 nm) between 17 and 20 °C at the concentration (c) (g/100 mL) indicated using, unless otherwise specified, spectroscopic grade chloroform as solvent. Measurements were carried out in a cell with a path length (l) of 1 dm. Low-resolution ESI mass spectra were recorded on a single quadrupole liquid chromatograph−mass spectrometer, while high-resolution measurements were conducted on a time-of-flight instrument. Low- and high-resolution EI mass spectra were recorded on a magnetic-sector machine. Melting points were measured on a Reichert melting point microscope and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates as supplied by Merck. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid/ceric sulfate/sulfuric acid (concn)/water (37.5 g: 7.5 g: 37.5 g: 720 mL) or potassium permanganate/potassium carbonate/5% sodium hydroxide aqueous solution/water (3 g: 20 g: 5 mL: 300 mL). Flash chromatographic separations were carried out following protocols defined by Still et al.[21] with silica gel 60 (40−63 μm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Starting materials and reagents were generally available from the Sigma-Aldrich, Merck, TCI, Strem, or Lancaster Chemical Companies and were used as supplied. Drying agents and other inorganic salts were purchased from the AJAX, BDH, or Unilab Chemical Companies. Tetrahydrofuran (THF), methanol, and dichloromethane were dried using a Glass Contour solvent purification system that is based upon a technology originally described by Grubbs et al.[22] Petroleum ether refers to the fraction boiling between 40 and 60 °C. Where necessary, reactions were performed under a nitrogen atmosphere.

*Specific Chemical Transformations. (1S,5R)-3-(2-Nitropyridin-3-yl)-6,8-dioxabicyclo- [3.2.1]oct-2-en-4-one (**9**).* A magnetically stirred solution of compound **3** (2.77 g, 11.0 mmol), pyridine **4** (1.0 g, 5.0 mmol), and copper(I) iodide (1.43 g, 7.5 mmol) containing copper powder (1.28 g, 20.0 mmol) and maintained at 50 °C was treated with Pd(dppf)Cl_2·CH_2Cl_2 (204 mg, 0.25 mmol). After 5 h, the reaction mixture was cooled to 22 °C, diluted with ethyl acetate (20 mL), and then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 40 mL) and the combined filtrates themselves washed with ammonia (2 × 25 mL of a 5% v/v aqueous solution), water (2 × 25 mL), then brine (1 × 25 mL) before being dried (Na_2SO_4) and filtered and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **9** (1.133 g, 91%) as a yellow, crystalline solid, mp = 170 °C, $[\alpha]_D$ = −140.0 (c 0.07, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (dd, J = 4.7 and 1.7 Hz, 1H), 7.79 (dd, J = 7.6 and 1.7 Hz, 1H), 7.65 (dd, J = 7.6 and 4.7 Hz, 1H), 7.32 (d, J = 4.7 Hz, 1H), 5.51 (s, 1H), 5.20 (m, 1H), 4.03 (m, 1H), 3.97 (d, J = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.2, 148.7, 144.3, 141.4, 134.6, 127.9, 123.3, 101.2, 72.1, 66.7 (one signal obscured or overlapping); IR (ATR) ν_{max} 2971, 1702, 1541, 1406, 1364, 1101, 984, 930, 890, 819, 647 cm^{-1}; MS (ESI, +ve) m/z 271 [(M + Na)$^+$, 100%], 249 [(M + H)$^+$, 29%]; HRMS (ESI, +ve) 249.0509 (M + H)$^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1S,5R)-3-(3-Nitropyridin-2-yl)-6,8-dioxabicyclo[3.2.1]oct-2-en-4-one (**10**).* A magnetically stirred mixture of compound **3** (1.50 g, 6.0 mmol), copper(I) iodide (857 mg, 4.5 mmol), copper powder (763 mg, 12.0 mmol), and Pd(dppf)Cl_2·CH_2Cl_2 (122 mg, 0.15 mmol) in dry DMSO (10 mL) maintained at 50 °C was treated, dropwise over 2 h, with a solution of pyridine **5** (609 mg, 3.0 mmol) in dimethyl sulfoxide (DMSO) (30 mL). After 4 h, the reaction mixture was cooled to 22 °C then diluted with ethyl acetate (20 mL) and water (20 mL) before being filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (3 × 20 mL) and the combined filtrates washed with water (2 × 30 mL) then brine (1 × 30 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **10** (589 mg, 79%) as a yellow, crystalline solid, no mp, decomposition above 120 °C, $[\alpha]_D$ = −231.7 (c 0.06, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (dd, J = 4.8 and 1.5 Hz, 1H), 8.34 (dd, J = 8.3 and 1.5 Hz, 1H), 7.72 (d, J = 4.8 Hz, 1H), 7.52 (m, 1H), 5.51 (s, 1H), 5.29 (m, 1H), 4.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.3, 153.1, 146.4,

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146.3, 146.1, 136.7, 132.3, 124.0, 101.2, 72.3, 66.6; IR (ATR) ν_{max} 3074, 2965, 2899, 1703, 1527, 1357, 1109, 1101, 984, 890, 574 cm^{-1}; MS (ESI, +ve) m/z 271 [$(M + Na)^+$, 100%]; HRMS (ESI, +ve) 249.0506 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1S,5R)-3-(3-Nitropyridin-4-yl)-6,8-dioxabicyclo[3.2.1]oct-2-en-4-one (**11**).* A magnetically stirred mixture of compound **3** (2.2 g, 8.8 mmol), pyridine **6** (812 mg, 4.0 mmol), copper(I) iodide (1.14 g, 6.0 mmol), and copper powder (1.27 g, 20.0 mmol) in dry DMSO (40 mL) maintained at 50 °C was treated with Pd(dppf)Cl_2·CH_2Cl_2 (34 mg, 0.04 mmol). After 5 h, the reaction mixture was cooled to 22 °C before being diluted with ethyl acetate (20 mL) then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 40 mL) and the combined filtrates washed with ammonia (2 × 25 mL of a 5% v/v aqueous solution), water (2 × 25 mL), and then brine (1 × 25 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **11** (348 mg, 35%) as a yellow, crystalline solid, no mp, decomposition above 90 °C, $[\alpha]_D$ = −268.3 (*c* 0.06, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 8.87 (d, *J* = 4.7 Hz, 1H), 7.36 (d, *J* = 4.7 Hz, 1H), 7.25 (s, 1H), 5.52 (s, 1H), 5.22 (s, 1H), 4.25-3.82 (complex m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.6, 154.1, 145.6, 144.4(2), 144.3(7), 136.6, 135.2, 125.2, 101.1, 72.1, 66.8; IR (ATR) ν_{max} 2983, 2901, 1712, 1544, 1524, 1355, 1107, 984, 935, 891, 579 cm^{-1}; MS (ESI, +ve) m/z 271 [$(M + Na)^+$, 100%]; HRMS (ESI, +ve) 249.0506 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1S,5R)-3-(6-Methyl-3-nitropyridin-2-yl)-6,8-dioxabicyclo-[3.2.1]oct-2-en-4-one (**12**).* A magnetically stirred mixture of compound **3** (717 mg, 2.8 mmol), pyridine **7** (308 mg, 1.4 mmol), copper(I) iodide (407 mg, 2.1 mmol), and copper powder (362 mg, 5.7 mmol) in dry DMSO (40 mL) maintained at 50 °C was treated with Pd(dppf)Cl_2·CH_2Cl_2 (23 mg, 0.03 mmol). After 5 h, the reaction mixture was cooled to 22 °C, diluted with ethyl acetate (20 mL), and then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 40 mL) and the combined filtrates washed with ammonia (2 × 25 mL of a 5% v/v aqueous solution), water (2 × 25 mL), and then brine (1 × 25 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **12** (207 mg, 56%) as a yellow, crystalline solid, no mp, decomposition above 105 °C, $[\alpha]_D$ = +18.3 (*c* 0.06, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 4.8 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 5.50 (s, 1H), 5.25 (m, 1H), 4.02 (broad s, 2H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.5, 163.6, 146.0, 145.8, 144.0, 137.0, 132.5, 123.6, 101.3, 72.3, 66.6, 24.7; IR (ATR) ν_{max} 3074, 2977, 2892, 1702, 1574, 1521, 1351, 1110, 985, 890, 834, 548 cm^{-1}; MS (ESI, +ve) m/z 285 [$(M + Na)^+$, 100%], 263 [$(M + H)^+$, 15]; HRMS (ESI, +ve) 263.0661 $(M + H)^+$ (calcd for $C_{12}H_{10}N_2O_5$ 263.0662).

*(1S,5R)-3-(5-Chloro-3-nitropyridin-2-yl)-6,8-dioxabicyclo-[3.2.1]oct-2-en-4-one (**13**).* A magnetically stirred mixture of compound **3** (1.5 g, 6.0 mmol), copper(I) iodide (857 mg, 4.5 mmol), copper powder (763 mg, 12.0 mmol), and Pd(dppf)-Cl_2·CH_2Cl_2 (122 mg, 0.15 mmol) in dry DMSO (10 mL) maintained at 50 °C was treated, dropwise over 2 h, with a solution of pyridine **8** (742 mg, 3.0 mmol) in DMSO (30 mL). After 4 h, the reaction mixture was cooled to 22 °C then diluted with ethyl acetate (20 mL) and water (20 mL) before being filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (3 × 20 mL) and the combined filtrates washed with water (2 × 30 mL) then brine (1 × 30 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **13** (699 mg, 82%) as a yellow, crystalline solid, no mp, decomposition above 150 °C, $[\alpha]_D$ = −416.0 (*c* 0.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 2.1 Hz, 1H), 8.34 (d, *J* = 2.1 Hz, 1H), 7.72 (d, *J* = 4.8 Hz, 1H), 5.50 (s, 1H), 5.27 (m, 1H), 4.06−3.98 (complex m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.0, 152.1, 146.9, 145.6, 144.2, 135.8, 132.3, 131.9, 101.1, 72.3, 66.6; IR (ATR) ν_{max} 3070, 2963, 2900, 1705, 1551, 1527, 1447, 1348, 1108, 984, 895, 440 cm^{-1}; MS (ESI, +ve) m/z 307 and 305 [$(M + Na)^+$, 35 and 100%]; HRMS (ESI, +ve) 283.0118 $(M + H)^+$ (calcd for $C_{11}H_7ClN_2O_5$ 283.0116).

*(6S,9R)-6,7,9,10-Tetrahydro-5H-6,9-epoxyoxepino-[4′,3′:4,5]pyrrolo[2,3-b]pyridine (**14**).* A magnetically stirred mixture of compound **9** (100 mg, 0.4 mmol) and 10% palladium on carbon (10 mg) in methanol (8 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 16 h, the reaction mixture was flushed with nitrogen then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **14** (19 mg, 23%) as a yellow, crystalline solid, no mp, decomposition above 130 °C, $[\alpha]_D$ = +11.1 (*c* 0.09, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (broad s, 1H), 8.31 (broad s, 1H), 7.83 (dd, *J* = 7.8 and 1.5 Hz, 1H), 7.09 (dd, *J* = 7.8 and 4.8 Hz, 1H), 6.28 (s, 1H), 5.02 (m, 1H), 4.12 (m, 1H), 3.71 (dd, *J* = 7.5 and 2.2 Hz, 1H), 3.43 (ddd, *J* = 15.8, 4.1 and 1.4 Hz, 1H), 2.60 (dd, *J* = 15.8 and 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 142.1, 134.6, 127.3, 119.8, 115.8, 102.7, 95.6, 72.8, 68.1, 28.8; IR (ATR) ν_{max} 3140, 3050, 2976, 2919, 2847, 2735, 1419, 1139, 1077, 946, 906, 767 cm^{-1}; MS (ESI, +ve) m/z 203 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 203.0812 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_2$ 203.0815).

*(6R,9S)-6,8,9,10-Tetrahydro-5H-6,9-epoxyoxepino-[4′,3′:4,5]pyrrolo[3,2-b]pyridine (**15**).* A magnetically stirred mixture of compound **10** (124 mg, 0.5 mmol) and 10% palladium on carbon (13 mg) in methanol (10 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, the reaction mixture was flushed with nitrogen then stirred at 50 °C for 16 h before being cooled to 22 °C then filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure and the residue thus obtained subjected to flash column chromatography (silica, 1:4 v/v acetone/dichloromethane elution). Concentration of the appropriate fractions (R_f = 0.5 in 1:1 v/v acetone/dichloromethane) gave compound **15** (104 mg, 95%) as a yellow,

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crystalline solid, no mp, decomposition above 150 °C, $[\alpha]_D$ = +52.0 (*c* 0.05, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 10.89 (broad s, 1H), 8.43 (dd, *J* = 4.7 and 1.4 Hz, 1H), 7.62 (dd, *J* = 8.2 and 1.4 Hz, 1H), 7.09 (m, 1H), 6.14 (s, 1H), 4.96 (broadened s, 1H), 4.04 (m, 1H), 3.68 (dd, *J* = 7.5 and 2.3 Hz, 1H), 3.46 (m, 1H), 2.69 (dd, *J* = 16.2 and 2.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.1, 142.7, 138.4, 129.2, 119.6, 117.0, 104.4, 95.5, 72.8, 68.2, 28.2; IR (ATR) ν_{max} 3051, 2971, 2898, 2843, 1359, 1136, 1080, 982, 882, 769, 579, 488 cm^{-1}; MS (ESI, +ve) *m*/*z* 203 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 203.0812 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_2$ 203.0815).

*(1R,4S)-3,4,5,10-Tetrahydro-1H-1,4-epoxyoxepino-[4′,3′:4,5]pyrrolo[2,3-c]pyridine (**16**).* A magnetically stirred mixture of compound **11** (124 mg, 0.5 mmol) and 10% palladium on carbon (13 mg) in methanol (10 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, the reaction mixture was flushed with nitrogen then stirred at 50 °C for 16 h before being cooled to 22 °C then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 5:95 v/v methanol/dichloromethane elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.5 in 10:90 v/v methanol/dichloromethane), compound **16** (94 mg, 93%) as a yellow, crystalline solid, mp = 173 °C, $[\alpha]_D$ = +8.0 (*c* 0.05, $CHCl_3$). ^1H NMR [400 MHz, $(CD_3)_2CO$] δ 8.81 (broad s, 1H), 8.20 (broad s, 1H), 7.46 (broad s, 1H), 6.25 (s, 1H), 5.02 (broadened s, 1H), 4.03 (m, 1H), 3.66 (dd, *J* = 7.6 and 2.2 Hz, 1H), 3.31 (ddd, *J* = 15.8, 4.2 and 1.5 Hz, 1H), 2.69 (d, *J* = 15.8 Hz, 1H) (signal due to N−H group proton not observed); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 138.1, 137.5, 134.6, 130.0, 114.5, 103.1, 94.5, 71.9, 67.6, 28.0; IR (ATR) ν_{max} 3045, 2966, 2906, 2846, 2725, 1704, 1449, 1365, 1136, 1082, 945, 810, 590 cm^{-1}; MS (ESI, +ve) *m*/*z* 203 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 203.0816 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_2$ 203.0815).

*(6R,9S)-2-Methyl-6,8,9,10-tetrahydro-5H-6,9-epoxyoxepino[4′,3′:4,5]pyrrolo[3,2-b]pyridine (**17**).* A magnetically stirred mixture of compound **12** (103 mg, 0.4 mmol) and 10% palladium on carbon (10 mg) in methanol (8 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, the reaction mixture was flushed with nitrogen then stirred at 50 °C for 16 h before being cooled to 22 °C then filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure and the residue thus obtained subjected to flash column chromatography (silica, 1:4 v/v acetone/dichloromethane elution). Concentration of the appropriate fractions (R_f = 0.5 in 1:1 v/v acetone/dichloromethane) then gave compound **17** (71 mg, 85%) as a white, crystalline solid, no mp, decomposition at ca. 180 °C, $[\alpha]_D$ = 20.0 (*c* 0.05, $CHCl_3$). ^1H NMR (400 MHz, CD_3OD) δ 7.67 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.03 (dd, *J* = 8.4 and 2.3 Hz, 1H), 6.17 (broad s, 1H), 5.00 (m, 1H), 4.04 (m, 1H), 3.65 (d, *J* = 7.5 Hz, 1H), 3.41-3.24 (complex m, 1H), 2.75 (broadened d, *J* = 16.0 Hz, 1H), 2.60 (s, 3H) (signal due to N−H group proton not observed); ^{13}C NMR (100 MHz, CD_3OD) δ 150.5, 143.0, 138.1, 127.5, 120.0, 116.6, 102.6, 95.4, 72.7, 67.7, 27.7, 22.2; IR (ATR) ν_{max} 3016, 2901, 2843, 2715, 1565, 1425, 1386, 1288, 1119, 1083, 888, 806 cm^{-1}; MS (ESI, +ve) *m*/*z* 217 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 217.0979 $(M + H)^+$ (calcd for $C_{12}H_{12}N_2O_2$ 217.0977).

*(6R,9R,10S)-3-Chloro-10-methoxy-6,8,9,10-tetrahydro-5H-6,9-epoxyoxepino[4′,3′:4,5]-pyrrolo-[3,2-b]pyridin-5-ol (**18**).* A magnetically stirred mixture of compound **13** (141 mg, 0.5 mmol) and 10% palladium on carbon (10 mg) in methanol (8 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, the reaction mixture was flushed with nitrogen then stirred at 50 °C for 16 h before being cooled to 22 °C and filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure and the residue thus obtained subjected to flash column chromatography (silica, 1:4 v/v acetone/dichloromethane elution). Concentration of the appropriate fractions (R_f = 0.5 in 1:1 v/v acetone/dichloromethane) then gave compound **18** (56 mg, 40%) as a white, crystalline solid, mp = 150 °C, $[\alpha]_D$ = +42.9 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 10.09 (broad s, 1H), 8.09 (d, *J* = 2.1 Hz, 1H), 7.16 (d, *J* = 2.1 Hz, 1H), 6.50 (s, 1H), 5.08 (m, 1H), 4.33 (m, 1H), 4.05 (m, 1H), 3.93 (s, 3H), 3.34 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.5, 136.7, 136.3, 125.8, 125.3, 116.2, 101.2, 98.9, 93.4, 74.2, 63.5, 58.2; IR (ATR) ν_{max} 3050, 2897, 1478, 1272, 1170, 1099, 1080, 1034, 983, 954, 892, 737 cm^{-1}; MS (ESI, +ve) *m*/*z* 589 and 587 [$(2M + Na)^+$, 100%], 285 and 283 [$(M + H)^+$, 30 and 80]; HRMS (ESI, +ve) 283.0475 $(M + H)^+$ (calcd for $C_{12}H_{11}ClN_2O_4$ 283.0480).

*(1R,5R)-3-Iodo-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**19**).* A magnetically stirred mixture of *iso*-LGO (**2**, 700 mg, 5.56 mmol) and pyridine (530 mg, 6.67 mmol) in dichloromethane (28 mL) maintained at 22 °C was treated with molecular iodine (1.83 g, 7.78 mmol). The ensuing mixture was stirred for 18 h then diluted with ethyl acetate (100 mL) and the resulting mixture washed with $Na_2S_2O_3$ (1 × 50 mL of a 1 M aqueous solution), water (1 × 100 mL), and brine (1 × 100 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 7:93 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **19** (911 mg, 66%) as a yellow, crystalline solid, mp = 112 °C, $[\alpha]_D$ = +158.8 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 3.5 Hz, 1H), 5.63 (d, *J* = 3.5 Hz, 1H), 5.01 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.13 (dd, *J* = 8.4 and 6.3 Hz, 1H), 3.66 (dd, *J* = 8.4 and 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.4, 155.6, 102.3, 97.5, 78.2, 62.9; IR (ATR) ν_{max} 3065, 2964, 2898, 2890, 1701, 1686, 1580, 1336, 1300, 1101, 979, 907, 524 cm^{-1}; MS (ESI, +ve) *m*/*z* 252 ($M^{+\bullet}$, 100%), 209 (17), 53 (22); HRMS (EI, 70 eV) 251.9289 ($M^{+\bullet}$) (calcd for $C_6H_5IO_3$ 251.9283).

*(1R,5R)-3-(2-Nitropyridin-3-yl)-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**21**).* A magnetically stirred mixture of compound **19** (126 mg, 0.5 mmol), pyridine **4** (121 mg, 0.6 mmol), copper(I) iodide (143 mg, 0.75 mmol), and copper powder (159 mg, 2.5 mmol) in dry DMSO (5 mL) maintained at 50 °C was treated with $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (8 mg, 0.01 mmol). After 5 h, the reaction mixture was cooled to 22 °C, diluted with ethyl acetate (10 mL), then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 20 mL) and the combined filtrates washed with ammonia (2 × 15 mL of a 5% v/v aqueous solution), water (2 × 15 mL), and then brine (1 × 15 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 1:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **21** (102 mg, 82%) as yellow, crystalline

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solid, no mp, decomposition above 120 °C, $[\alpha]_D$ = −20.0 (*c* 0.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (dd, *J* = 4.6 and 1.7 Hz, 1H), 7.78 (dd, *J* = 7.6 and 1.7 Hz, 1H), 7.67 (dd, *J* = 7.6 and 4.6 Hz, 1H), 7.17 (d, *J* = 3.4 Hz, 1H), 5.96 (d, *J* = 3.4 Hz, 1H), 4.89 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.17 (dd, *J* = 8.4 and 6.3 Hz, 1H), 3.90 (dd, *J* = 8.4 and 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9, 156.4, 148.8, 143.7, 141.5, 135.1, 128.5, 123.2, 96.5, 79.1, 62.5; IR (ATR) ν_{max} 3063, 2964, 2898, 1709, 1538, 1405, 1359, 1116, 986, 915, 730, 645, 533 cm^{-1}; MS (ESI, +ve) *m*/*z* 271 [$(M + Na)^+$, 100%]; HRMS (ESI, +ve) 249.0506 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1R,5R)-3-(3-Nitropyridin-2-yl)-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**22**).* A magnetically stirred mixture of compound **19** (126 mg, 0.5 mmol), copper(I) iodide (143 mg, 0.75 mmol), copper powder (159 mg, 2.5 mmol), and Pd(dppf)Cl_2·CH_2Cl_2 (8 mg, 0.01 mmol) in dry DMSO (2 mL) maintained at 50 °C was treated, dropwise over 2 h, with the solution of pyridine **5** (121 mg, 0.6 mmol) in DMSO (3 mL). After 4 h, the reaction mixture was cooled to 22 °C, diluted with ethyl acetate (10 mL) and water (10 mL), and then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (3 × 10 mL) and the combined filtrates washed with water (2 × 10 mL) then brine (1 × 10 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 1:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **22** (105 mg, 84%) as a yellow, crystalline solid, no mp, decomposition above 120 °C, $[\alpha]_D$ = +52.5 (*c* 0.04, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (dd, *J* = 4.7 and 1.5 Hz, 1H), 8.36 (dd, *J* = 8.3 and 1.5 Hz, 1H), 7.78-7.40 (complex m, 2H), 6.03 (d, *J* = 3.4 Hz, 1H), 4.91 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.19 (dd, *J* = 8.4 and 6.3 Hz, 1H), 3.93 (dd, *J* = 8.4 and 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 153.3, 146.1, 145.8, 136.9, 132.3, 124.2, 96.7, 79.2, 62.5 (one signal obscured or overlapping); IR (ATR) ν_{max} 2967, 1702, 1593, 1526, 1356, 1114, 986 cm^{-1}; MS (ESI, +ve) *m*/*z* 271 [$(M + Na)^+$, 100%]; HRMS (ESI, +ve) 249.0506 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1R,5R)-3-(3-Nitropyridin-4-yl)-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**23**).* A magnetically stirred mixture of compound **19** (166 mg, 0.66 mmol), pyridine **7** (160 mg, 0.79 mmol), copper(I) iodide (190 mg, 1 mmol), and copper powder (209 mg, 3.3 mmol) in dry DMSO (7 mL) maintained at 22 °C was treated with Pd(dppf)Cl_2·CH_2Cl_2 (27 mg, 0.03 mmol). After 16 h, the reaction mixture was diluted with ethyl acetate (10 mL) then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 20 mL) and the combined filtrates washed with ammonia (2 × 15 mL of a 5% v/v aqueous solution), water (2 × 15 mL), and then brine (1 × 15 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **23** (84 mg, 51%) as a yellow, crystalline solid, no mp, decomposition above ca. 90 °C, $[\alpha]_D$ = −15.0 (*c* 0.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.87 (broad s, 1H), 7.25 (d, *J* = 4.9 Hz, 1H), 7.20 (d, *J* = 3.4 Hz, 1H), 5.99 (d, *J* = 3.4 Hz, 1H), 4.92 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.20 (dd, *J* = 8.4 and 6.3 Hz, 1H), 3.95 (dd, *J* = 8.4 and 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.3, 154.4, 145.7, 143.9, 136.2, 135.5, 125.1, 96.4, 79.2, 62.6 (one signal obscured or overlapping); IR (ATR) ν_{max} 3058, 2965, 1708, 1598, 1544, 1524, 1359, 1110, 1033, 987, 918, 856, 583, 533 cm^{-1}; MS (ESI, +ve) *m*/*z* 303 [$(M + Na + MeOH)^+$, 100%]; HRMS (ESI, +ve) 249.0505 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(1R,5R)-3-(6-Methyl-3-nitropyridin-2-yl)-6,8-dioxabicyclo-[3.2.1]oct-3-en-2-one (**24**).* A magnetically stirred mixture of compound **19** (252 mg, 1.0 mmol), copper(I) iodide (286 mg, 1.5 mmol), copper powder (312 mg, 5.0 mmol), and Pd(dppf)Cl_2·CH_2Cl_2 (40 mg, 0.05 mmol) in dry DMSO (4 mL) maintained at 50 °C was treated, dropwise over 2 h, with a solution of pyridine **7** (260 mg, 1.2 mmol) in DMSO (6 mL). After 4 h, the reaction mixture was cooled to 22 °C, diluted with ethyl acetate (10 mL) then filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 20 mL) and the combined filtrates washed with ammonia (2 × 15 mL of a 5% v/v aqueous solution), water (2 × 15 mL), and then brine (1 × 15 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **24** (191 mg, 73%) as a white, crystalline solid, no mp, decomposition above ca. 115 °C, $[\alpha]_D$ = +6.3 (*c* 0.08, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 3.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 6.01 (d, *J* = 3.4 Hz, 1H), 4.88 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.17 (dd, *J* = 8.3 and 6.3 Hz, 1H), 3.94 (dd, *J* = 8.3 and 1.5 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 163.8, 145.5, 145.4, 144.1, 137.2, 132.5, 123.8, 96.7, 79.2, 62.5, 24.7; IR (ATR) ν_{max} 3079, 2996, 2898, 1702, 1573, 1519, 1349, 1118, 986, 922, 736, 535 cm^{-1}; MS (ESI, +ve) *m*/*z* 285 [$(M + Na)^+$, 100%]; HRMS (ESI, +ve) 263.0664 $(M + H)^+$ (calcd for $C_{12}H_{10}N_2O_5$ 263.0662).

*(1R,5R)-3-(5-Chloro-3-nitropyridin-2-yl)-6,8-dioxabicyclo-[3.2.1]oct-3-en-2-one (**25**).* A magnetically stirred mixture of compound **19** (252 mg, 1.0 mmol), copper(I) iodide (286 mg, 1.5 mmol), copper powder (312 mg, 5.0 mmol), and Pd(dppf)Cl_2·CH_2Cl_2 (40 mg, 0.05 mmol) in dry DMSO (4 mL) maintained at 50 °C was treated, dropwise over 2 h, with a solution of pyridine **8** (298 mg, 1.25 mmol) in DMSO (6 mL). After 4 h, the reaction mixture was cooled to 22 °C then diluted with ethyl acetate (10 mL) and water (10 mL) before being filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (3 × 15 mL) and the combined filtrates washed with water (2 × 15 mL) then brine (1 × 15 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 1:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **25** (156 mg, 55%) as a yellow, crystalline solid, no mp, decomposition above ca. 110 °C, $[\alpha]_D$ = +46.0 (*c* 0.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.2 Hz, 1H), 8.35 (d, *J* = 2.2 Hz, 1H), 7.53 (d, *J* = 3.4 Hz, 1H), 6.03 (d, *J* = 3.4 Hz, 1H), 4.91 (dd, *J* = 6.3 and 1.5 Hz, 1H), 4.19 (dd, *J* = 8.4 and 6.3 Hz, 1H), 3.90 (dd, *J* = 8.4 and 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9, 152.3, 146.3, 145.7, 143.9, 136.0, 132.5,

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131.9, 96.6, 79.2, 62.5; IR (ATR) ν_{max} 3070, 2899, 1705, 1551, 1528, 1448, 1350, 1114, 986, 899, 776, 563 cm^{-1}; MS (ESI, +ve) m/z 307 and 305 [$(M + Na)^+$, 35 and 100%]; HRMS (ESI, +ve) 283.0118 $(M + H)^+$ (calcd for $C_{11}H_7ClN_2O_5$ 283.0116).

*(1R,5R)-3-(4-Nitropyridin-3-yl)-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**26**).* A magnetically stirred mixture of compound **19** (170 mg, 0.67 mmol), pyridine **20** (137 mg, 0.67 mmol), copper(I) iodide (190 mg, 1.0 mmol), and copper powder (214 mg, 3.37 mmol) in dry DMSO (7 mL) maintained at 50 °C was treated with $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (5 mg, 0.06 mmol). After 5 h, the reaction mixture was cooled to 22 °C then diluted with ethyl acetate (10 mL) before being filtered through a plug of TLC-grade silica topped with diatomaceous earth. The solids so retained were washed with ethyl acetate (1 × 20 mL) and the combined filtrates washed with ammonia (2 × 15 mL of a 5% v/v aqueous solution), water (2 × 15 mL), and then brine (1 × 15 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **26** (78 mg, 46%) as a yellow, crystalline solid, no mp, decomposition above 105 °C, $[\alpha]_D$ = −52.0 (*c* 0.05, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (broad s, 1H), 8.69 (broad s, 1H), 7.89 (s, 1H), 7.22 (d, J = 3.3 Hz, 1H), 5.98 (d, J = 3.3 Hz, 1H), 4.89 (m, 1H), 4.17 (dd, J = 8.5 and 6.3 Hz, 1H), 3.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 153.8, 152.5, 152.2, 143.8, 134.0, 122.1, 117.1, 96.5, 79.1, 62.4; IR (ATR) ν_{max} 3098, 3059, 2965, 1709, 1529, 1355, 1108, 986, 913, 858, 720, 535 cm^{-1}; MS (ESI, +ve) m/z 303 [$(M + Na + MeOH)^+$, 100%], 271 [$(M + Na)^+$, 60]; HRMS (ESI, +ve) 249.0506 $(M + H)^+$ (calcd for $C_{11}H_8N_2O_5$ 249.0506).

*(6R,9S)-5,8,9,10-Tetrahydro-6H-6,9-epoxyoxepino-[4′,5′:4,5]pyrrolo[2,3-b]pyridine (**27**).* A magnetically stirred mixture of compound **21** (194 mg, 0.78 mmol) and 10% palladium on carbon (20 mg) in methanol (16 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, acetic acid (47 μL, 0.78 mmol) was added to the reaction mixture that was then flushed with nitrogen before being stirred at 50 °C for 16 h. After this time, the mixture was cooled to 22 °C then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, ethyl acetate elution) and so afforded, after concentration of the appropriate fractions (R_f = 0.5 in 1:99 v/v methanol/ethyl acetate), compound **27** (75 mg, 48%) as a white, crystalline solid, no mp, decomposition above ca. 160 °C, $[\alpha]_D$ = −21.3 (*c* 0.08, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 12.41 (broadened s, 1H), 8.23 (dd, J = 4.8 and 1.5 Hz, 1H), 7.78 (dd, J = 7.8 and 1.5 Hz, 1H), 7.07 (dd, J = 7.8 and 4.8 Hz, 1H), 6.04 (d, J = 2.4 Hz, 1H), 5.43 (d, J = 4.1 Hz, 1H), 4.13 (d, J = 6.7 Hz, 1H), 4.02 (dd, J = 6.7 and 4.1 Hz, 1H), 3.16 (ddd, J = 15.7, 2.4 and 1.1 Hz, 1H), 2.79 (d, J = 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 141.1, 135.4, 127.0, 120.5, 115.7, 102.3, 101.2, 73.7, 70.5, 30.7; IR (ATR) ν_{max} 3143, 3057, 2940, 2886, 1587, 1417, 1288, 1144, 1001, 996, 768, 514 cm^{-1}; MS (ESI, +ve) m/z 203 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 203.0822 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_2$ 203.0821).

*(6S,9R)-6,7,9,10-Tetrahydro-5H-6,9-epoxyoxepino-[4′,5′:4,5]pyrrolo[3,2-b]pyridine (**28**).* A magnetically stirred mixture of compound **22** (170 mg, 0.68 mmol) and 10% palladium on carbon (17 mg) in methanol (14 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, acetic acid (40 μL, 0.68 mmol) was added to the reaction mixture that was flushed with nitrogen before being stirred at 50 °C for 16 h. After this time, the reaction mixture was cooled to 22 °C then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, ethyl acetate elution) and so afforded, after concentration of the appropriate fractions (R_f = 0.5 in 5:95 v/v methanol/ ethyl acetate), compound **28** (123 mg, 89%) as a yellow oil, $[\alpha]_D$ = −30.2 (*c* 0.04, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 10.87 (broadened s, 1H), 8.33 (d, J = 4.8 Hz, 1H), 7.67 (dd, J = 8.2 and 1.4 Hz, 1H), 7.07 (ddd, J = 8.2, 4.8 and 1.3 Hz, 1H), 5.94 (d, J = 2.3 Hz, 1H), 5.28 (d, J = 4.2 Hz, 1H), 4.02 (d, J = 6.6 Hz, 1H), 3.88 (dd, J = 6.6 and 4.2 Hz, 1H), 3.13 (dd, J = 16.0 and 2.3 Hz, 1H), 2.85 (d, J = 16.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.9, 141.3, 139.9, 129.5, 120.0, 116.5, 103.4, 101.0, 73.4, 70.4, 30.3; IR (ATR) ν_{max} 3138, 3051, 2954, 2888, 1556, 1412, 1359, 1106, 999, 845, 729, 517 cm^{-1}; MS (ESI, +ve) m/z 203 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 203.0822 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_2$ 203.0821).

*(6R,9S)-5,6,8,9-Tetrahydro-10H-6,9-epoxyoxepino-[4′,5′:4,5]pyrrolo[2,3-c]pyridin-10-ol (**29**).* A magnetically stirred mixture of compound **23** (87 mg, 0.35 mmol) and 10% palladium on carbon (9 mg) in methanol (7 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 2 h, acetic acid (20 μL, 0.35 mmol) was added to the reaction mixture that was flushed with nitrogen then stirred at 50 °C for 16 h. After this time, the reaction mixture was cooled to 22 °C then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, ethyl acetate elution) and so afforded, after concentration of the appropriate fractions (R_f = 0.1 in 1:4 v/v methanol/dichloromethane), compound **29** (42 mg, 55%) as a yellow, crystalline solid, no mp, decomposition above ca. 150 °C, $[\alpha]_D$ = +31.4 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 13.91 (broadened s, 1H), 8.58 (m, 1H), 7.75 (s, 1H), 7.34 (m, 1H), 6.00 (d, J = 2.3 Hz, 1H), 5.72 (d, J = 3.9 Hz, 1H), 4.10 (m, 2H), 3.10 (dd, J = 15.7 and 2.5 Hz, 1H), 2.78 (d, J = 15.7 Hz, 1H); ^{13}C NMR [100 MHz, $(CD_3)_2CO$] δ 139.9, 134.6, 130.0, 127.4, 113.4, 104.3, 100.7, 99.0, 72.9, 68.2, 30.1; IR (ATR) ν_{max} 3062, 2955, 2896, 1637, 1611, 1440, 1111, 1026, 954, 858, 825, 737 cm^{-1}; MS (ESI, +ve) m/z 219 [$(M + H)^+$, 100%]; HRMS (ESI, +ve) 219.0761 $(M + H)^+$ (calcd for $C_{11}H_{10}N_2O_3$ 219.0764).

*(6S,9R)-2-Methyl-6,7,9,10-tetrahydro-5H-6,9-epoxyoxepino[4′,5′:4,5]pyrrolo[3,2-b]pyridine (**30**).* A magnetically stirred mixture of compound **24** (194 mg, 0.74 mmol) and 10% palladium on carbon (19 mg) in methanol (15 mL) maintained at 22 °C was placed under a balloon of hydrogen. After 24 h, the reaction mixture was filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1:99 v/v methanol/ ethyl acetate elution) and so afforded, after concentration of the appropriate fractions (R_f = 0.5 in 1:9 v/v methanol/ethyl acetate), compound **30** (136 mg, 85%) as a yellow, crystalline solid, no mp, decomposition above ca. 128 °C, $[\alpha]_D$ = −52.0 (*c* 0.05, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 11.05 (broad

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s, 1H), 7.51 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 5.90 (d, J = 2.4 Hz, 1H), 5.19 (s, 1H), 3.98 (d, J = 6.6 Hz, 1H), 3.84 (m, 1H), 3.08 (dd, J = 16.0 and 2.4 Hz, 1H), 2.83 (d, J = 16.0 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2, 144.4, 138.3, 127.1, 119.2, 116.8, 103.8, 101.2, 73.5, 70.4, 30.3, 24.1; IR (ATR) ν_{max} 2954, 2920, 1567, 1423, 1291, 1107, 1055, 1002, 845, 734, 525 cm^{-1}; MS (ESI, +ve) m/z 217 [(M + H)$^+$, 100%]; HRMS (ESI, +ve) 217.0979 (M + H)$^+$ (calcd for $C_{12}H_{12}N_2O_2$ 217.0977).

*(5S,6R,9R)-6,7,9,10-Tetrahydro-5H-6,9-epoxyoxepino-[4′,3′:4,5]pyrrolo[2,3-b]pyridin-5-ol (**31**).* A magnetically stirred mixture of compound **9** (50 mg, 0.2 mmol) and iron powder (56 mg, 1 mmol) in dimethyl ether (DME) (1 mL) was treated with acetic acid (1 mL of a 1 M aqueous solution) then heated at 50 °C for 8 h before being cooled and filtered through a pad of diatomaceous earth. The filtrate was diluted was ethyl acetate (50 mL) then washed with water (1 × 20 mL) and brine (1 × 20 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 3:1 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **31** (29 mg, 68%) as white, crystalline solid, no mp, decomposition above ca. 130 °C, $[\alpha]_D$ = −124.3 (c 0.07, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.26 (broad s, 1H), 8.10 (dd, J = 7.9 and 1.5 Hz, 1H), 8.02 (dd, J = 4.8 and 1.5 Hz, 1H), 7.15 (dd, J = 7.9 and 4.8 Hz, 1H), 5.65 (s, 1H), 4.96 (ddd, J = 6.7, 2.3 and 1.5 Hz, 1H), 4.53 (d, J = 1.5 Hz, 1H), 4.01 (dd, J = 8.0 and 6.7 Hz, 1H), 3.39 (dd, J = 8.0 and 2.3 Hz, 1H) (signal due to OH group proton not observed); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.1, 142.3, 134.9, 128.2, 119.0, 116.3, 105.1, 94.9, 79.1, 66.4, 63.8; IR (ATR) ν_{max} 3153, 3054, 2977, 1557, 1418, 1284, 1145, 1080, 983, 942, 894, 773, 535 cm^{-1}; MS (ESI, +ve) m/z 219 [(M + H)$^+$, 100%]; HRMS (ESI, +ve) 219.0771 (M + H)$^+$ (calcd for $C_{11}H_{10}N_2O_3$ 219.0770).

*(5S,6R,9R)-5-Ethoxy-6,7,9,10-tetrahydro-5H-6,9-epoxyoxepino[4′,3′:4,5]pyrrolo[2,3-b]pyridine (**32**).* A magnetically stirred mixture of compound **9** (124 mg, 0.5 mmol), $CaCl_2$ (175 mg, 1.5 mmol), and iron powder (84 mg, 1.5 mmol) in water/ethanol (5 mL of a 1:4 v/v mixture) was heated under reflux for 2 h. The cooled reaction mixture was filtered through a pad of diatomaceous earth and the filtrate diluted was ethyl acetate (50 mL) before being washed with water (1 × 20 mL) and brine (1 × 20 mL) then dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 1:3 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **32** (67 mg, 55%) as a white, crystalline solid, no mp, decomposition above ca. 125 °C, $[\alpha]_D$ = −140.0 (c 0.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 8.34 (broad s, 1H), 7.98 (dd, J = 7.9 and 1.4 Hz, 1H), 7.15 (m, 1H), 6.31 (s, 1H), 5.07 (m, 1H), 4.40 (s, 1H), 4.08 (dd, J = 7.8 and 6.7 Hz, 1H), 3.93-3.60 (complex m, 2H), 3.47 (dd, J = 7.8 and 2.3 Hz, 1H), 1.29 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 142.5, 136.1, 128.1, 119.8, 116.6, 103.4, 95.5, 76.3, 73.1, 64.1(0), 64.0(6), 15.7; IR (ATR) ν_{max} 3150, 3051, 2974, 2893, 1555, 1416, 1280, 1147, 1078, 984, 889, 773 cm^{-1}; MS (ESI, +ve) m/z 269 [(M + Na)$^+$, 100%], 247 [(M + H)$^+$, 11]; HRMS (ESI, +ve) 247.1083 (M + H)$^+$ (calcd for $C_{13}H_{14}N_2O_3$ 247.1083).

*(6R,9S)-9,10-Dihydro-6H-6,9-epoxyoxepino[4′,5′:4,5]-pyrrolo[2,3-b]pyridin-5(8H)-one (**33**).* A magnetically stirred mixture of compound **9** (50 mg, 0.2 mmol) and iron powder (56 mg, 1 mmol) in DME (1 mL) was treated with HCl (1 mL of a 1 M of an aqueous solution) then heated at 50 °C for 18 h before being cooled then filtered through a pad of diatomaceous earth. The filtrate was diluted was ethyl acetate (50 mL) before being washed with water (1 × 20 mL) and brine (1 × 20 mL) then dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue so obtained was subjected to flash column chromatography (silica, 2:1 v/v ethyl acetate/40−60 petroleum ether elution) and thus afforded, after concentration of the appropriate fractions (R_f = 0.2), compound **33**[11b] (26 mg, 58%) as white, crystalline solid, mp = 275 °C, $[\alpha]_D$ = −221.7 (c 0.06, $CHCl_3$). ^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 11.59 (broad s, 1H), 8.37 (broadened s, 1H), 8.25 (dd, J = 7.8 and 1.6 Hz, 1H), 7.29 (dd, J = 7.8 and 4.6 Hz, 1H), 5.95 (d, J = 4.8 Hz, 1H), 5.44 (s, 1H), 4.11 (dd, J = 7.2 and 4.6 Hz, 1H), 3.96 (d, J = 7.2 Hz, 1H) (signal due to N−H group proton not observed); ^{13}C NMR [100, MHz, $(CD_3)_2CO$] δ 185.6, 151.3, 148.9, 144.8, 128.7, 118.6, 116.5, 106.2, 102.3, 71.2, 67.1; IR (ATR) ν_{max} 2981, 2888, 1679, 1592, 1480, 1416, 1109, 1075, 889, 805 cm^{-1}; MS (ESI, +ve) m/z 239 [(M + Na)$^+$, 100%], 217 [(M + H)$^+$, 10]; HRMS (ESI, +ve) 217.0609 (M + H)$^+$ (calcd for $C_{11}H_8N_2O_3$ 217.0608).

*Formation of L-Cysteine Adducts with Compounds **9**−**12**, **14**−**17** and **21**−**30** for Mass Spectral Analysis.* A magnetically stirred solution of the relevant compound (0.01 mmol) in methanol (0.2 mL) maintained at 22 °C was treated with L-cysteine (1.4 mg, 0.01 mmol) or the equivalent amount of the corresponding methyl ester and after 24 h the reaction mixture was diluted with methanol (2 mL) then analyzed using both +ve and −ve ionization modes of ESI mass spectrometry.

*S-((1R,2S,3R,5R)-3-(3-Nitropyridin-2-yl)-4-oxo-6,8-dioxabicyclo[3.2.1]octan-2-yl)-L-cysteine (**34**) and S-((1R,2S,3S,5R)-3-(3-Nitropyridin-2-yl)-4-oxo-6,8-dioxabicyclo[3.2.1]octan-2-yl)-L-cysteine (**35**).* A magnetically stirred solution of compound **10** (100 mg, 0.4 mmol) in methanol (1.4 mL) maintained at 22 °C was treated with a solution of L-cysteine (24 mg, 0.2 mmol) in water (700 μL). After 48 h the resulting mixture was diluted with methanol (10 mL) then filtered through a pad of diatomaceous earth and the filtrate concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1:10:10 v/v/v acetic acid/methanol/ethyl acetate elution) and thus afforded, two fractions, A and B.

Concentration of fraction A (R_f = 0.3 in 1:10:10 v/v/v acetic acid/methanol/ethyl acetate) afforded compound **34** (13 mg, 18%) as a yellow oil, $[\alpha]_D$ = −93.3 (c 0.03, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (dd, J = 4.7 and 1.4 Hz, 1H), 8.30 (dd, J = 8.3 and 1.4 Hz, 1H), 7.46 (dd, J = 8.3 and 4.7 Hz, 1H), 5.28 (s, 1H), 4.31 (m, 1H), 4.20 (m, 1H), 3.84-3.70 (complex m, 2H), 3.56 (m, 1H), 3.30 (m, 1H), 3.09 (dd, J = 14.7 and 3.7 Hz, 1H), 2.74 (dd, J = 14.7 and 9.8 Hz, 1H) (signals due to NH_2 and CO_2H group protons not observed); ^{13}C NMR (100 MHz, CD_3OD) δ 168.7, 152.4, 152.1, 146.9, 132.9, 122.8, 98.6, 80.6(4), 80.6(1), 68.3, 54.4, 51.8, 36.4, 33.7; IR (ATR) ν_{max} 3384, 2925, 1704, 1595, 1565, 1525, 1407, 1347, 1279, 1107, 1013, 818, 617, 455 cm^{-1}; MS (ESI, +ve) m/z 424 [(M+CH_3OH+Na)$^+$, 100%]; HRMS (ESI, +ve) 402.0956 (M+CH_3OH+H)$^+$ (calcd for $C_{14}H_{15}N_3O_7S$ 402.0966).

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Concentration of fraction B (R_f = 0.2 in 1:10:10 v/v/v acetic acid/methanol/ethyl acetate) afforded compound **35** (28 mg, 38%) as a yellow oil, $[\alpha]_D$ = −60.0 (*c* 0.02, CH_3OH). ^1H NMR (400 MHz, D_2O) δ 8.80 (dd, *J* = 4.8 and 1.5 Hz, 1H), 8.52 (dd, *J* = 8.4 and 1.5 Hz, 1H), 7.64 (dd, *J* = 8.4 and 4.8 Hz, 1H), 5.18 (s, 1H), 4.40 (q, *J* = 6.3 Hz, 1H), 4.18 (dd, *J* = 8.5 and 6.8 Hz, 1H), 4.00 (dd, *J* = 8.5 and 6.4 Hz, 1H), 3.88 (m, 1H), 3.75−3.39 (complex m, 2H), 3.01−2.89 (complex m, 2H) (signals due to NH_2 and CO_2H group protons not observed); ^{13}C NMR (100 MHz, D_2O) δ 173.9, 172.3, 152.6, 152.2, 146.5, 134.4, 123.7, 100.1, 79.2, 68.0, 54.0, 48.9, 37.1, 32.8; IR (ATR) ν_{max} 3408, 2927, 1704, 1595, 1564, 1525, 1407, 1347, 1279, 1107, 1013, 807, 617, 454 cm^{-1}. Satisfactory MS and HRMS data could not be obtained on this compound.

Crystallographic Studies. *Crystallographic Data. Compound* ***10***. $C_{11}H_8N_2O_5$, *M* = 248.19, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 9.6892(4) Å, *b* = 9.8837(4) Å, *c* = 10.8874(4) Å; *V* = 1042.63(7) Å^3, D_x = 1.581 Mg m^{-3}, 2443 unique data ($2\theta_{max}$ = 58.33°), *R* = 0.0370 [for 2263 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0831 (all data), *S* = 1.053.

Compound ***13***. $C_{11}H_7ClN_2O_5$, *M* = 282.64, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 7.9009(2) Å, *b* = 10.9614(3) Å, *c* = 13.2772(4) Å; *V* = 1149.87(6) Å^3, D_x = 1.633 Mg m^{-3}, 2681 unique data ($2\theta_{max}$ = 58.444°), *R* = 0.0360 [for 2483 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0764 (all data), *S* = 1.037.

Compound ***14***. $C_{11}H_{10}N_2O_2$, *M* = 202.21, *T* = 150 K, monoclinic, space group $P2_1$, *Z* = 8, *a* = 9.90829(8) Å, *b* = 9.74229(7) Å, *c* = 19.67523(17) Å; α = 90°, β = 102.5485(8)°, γ = 90°; *V* = 1853.87(3) Å^3, D_x = 1.449 Mg m^{-3}, 7371 unique data ($2\theta_{max}$ = 147.8°), *R* = 0.036 [for 7251 reflections with $I > 2.0\sigma(I)$]; R_w = 0.097 (all data), *S* = 1.04.

Compound ***16***. $C_{11}H_{10}N_2O_6$, *M* = 202.21, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 5.12226(4) Å, *b* = 9.87199(79) Å, *c* = 18.24358(17) Å; α = 90°, β = 90°, γ = 90°; *V* = 922.52(2) Å^3, D_x = 1.456 Mg m^{-3}, 1875 unique data ($2\theta_{max}$ = 147.8°), *R* = 0.023 [for 1860 reflections with $I > 2.0\sigma(I)$]; R_w = 0.063 (all data), *S* = 1.06.

Compound ***17***. $C_{12}H_{12}N_2O_2$, *M* = 216.24, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 8, *a* = 7.82892(6) Å, *b* = 11.64835(9) Å, *c* = 23.20164(18) Å; α = 90°, β = 90°, γ = 90°; *V* = 2115.85(3) Å^3, D_x = 1.358 Mg m^{-3}, 4293 unique data ($2\theta_{max}$ = 147.6°), *R* = 0.026 [for 4199 reflections with $I > 2.0\sigma(I)$]; R_w = 0.069 (all data), *S* = 1.03.

Compound ***22***. $C_{11}H_8N_2O_5$, *M* = 248.19, *T* = 150 K, monoclinic, space group $P2_1$, *Z* = 2, *a* = 5.6724(2) Å, *b* = 9.6556(3) Å, *c* = 9.4591(4) Å; β = 103.910(4)°; *V* = 502.89(3) Å^3, D_x = 1.639 Mg m^{-3}, 2450 unique data ($2\theta_{max}$ = 58.478°), *R* = 0.0347 [for 2314 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0778 (all data), *S* = 1.072.

Compound ***23***. $C_{11}H_8N_2O_5$, *M* = 248.19, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 10.0284(3) Å, *b* = 11.8376(3) Å, *c* = 18.0484(5) Å; *V* = 2142.57(10) Å^3, D_x = 1.539 Mg m^{-3}, 5260 unique data ($2\theta_{max}$ = 58.734°), *R* = 0.0390 [for 4752 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0823 (all data), *S* = 1.071.

Compound ***29***. $C_{11}H_{11}N_2O_{3.5}$, *M* = 227.22, *T* = 150 K, trigonal, space group $P3_221$, *Z* = 6, *a* = 14.9944(3) Å, *b* = 14.9944(3) Å, *c* = 9.21720(10) Å; α = 90°, β = 90°, γ = 120°; *V* = 1794.68(7) Å^3, D_x = 1.261 Mg m^{-3}, 2443 unique data ($2\theta_{max}$ = 147.48°), *R* = 0.0311 [for 2394 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0962 (all data), *S* = 1.133.

Compound ***30***. $C_{13}H_{14}Cl_2N_2O_2$, *M* = 301.16, *T* = 150 K, triclinic, space group *P*1, *Z* = 2, *a* = 8.3394(3) Å, *b* = 9.2251(3) Å, *c* = 10.2483(3) Å; α = 112.584(3)°, β = 102.063(3)°, γ = 99.352(3)°; *V* = 685.67(4) Å^3, D_x = 1.459 Mg m^{-3}, 4429 unique data ($2\theta_{max}$ = 147.6°), *R* = 0.031 [for 4363 reflections with $I > 2.0\sigma(I)$]; R_w = 0.087 (all data), *S* = 1.05.

Compound ***32***. $C_{13}H_{14}N_2O_3$, *M* = 246.26, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 9.26094(4) Å, *b* = 9.56903(5) Å, *c* = 13.38456(6) Å; α = 90°, β = 90°, γ = 90°; *V* = 1186.12(1) Å^3, D_x = 1.379 Mg m^{-3}, 2405 unique data ($2\theta_{max}$ = 147.6°), *R* = 0.041 [for 2393 reflections with $I > 2.0\sigma(I)$]; R_w = 0.120 (all data), *S* = 1.19.

Compound ***33***. $C_{11}H_8N_2O_6$, *M* = 216.19, *T* = 150 K, orthorhombic, space group $P2_12_12_1$, *Z* = 4, *a* = 4.7466(1) Å, *b* = 12.197927 Å, *c* = 16.5531(3) Å; α = 90°, β = 90°, γ = 90°; *V* = 958.40(3) Å^3, D_x = 1.498 Mg m^{-3}, 1931 unique data ($2\theta_{max}$ = 148.182°), *R* = 0.0313 [for 1846 reflections with $I > 2.0\sigma(I)$]; R_w = 0.0831 (all data), *S* = 1.063.

Structure Determinations. Data for compounds **14**, **16**, **17**, **29**, **30**, **32**, and **33** were measured on a Rigaku SuperNova diffractometer using Cu Kα, graphite monochromator (λ = 1.54184 Å), while those for compounds **10**, **13**, **22**, and **23** were recorded using Mo Kα, graphite monochromator (λ = 0.71073 Å). Data collection, cell refinement, and data reduction employed the CrysAlis PRO program,[23] while SHELXT[24] and SHELXL[25] were used for structure solution and refinement. Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC nos. 1990200−1990210). These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Accession Codes. CCDC depositions 1990200−1990210 contain the supplementary crystallographic data for this article. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033.

Biological Studies. *Antibacterial Assays.* The bacterium to be tested was streaked onto a tryptic soy agar plate then incubated at 37 °C for 24 h. One colony was then transferred to fresh tryptic soy broth (15 mL) and the cell density was adjusted to 10^4−10^5 CFU/mL. The compounds to be tested were dissolved in DMSO and diluted with H_2O to give 600 μM stock solution (20% DMSO). For the single data point experiment, an aliquot (10 μL) from 600 μM stock solution was transferred to a 96-well microtiter plate, and freshly prepared microbial broth (190 μL) was added to each well to give final concentrations of 30 μM in 1% DMSO. For the serial dilution experiments, the 600 μM stock solution was serially diluted with 20% DMSO to give concentrations from 600 to 0.2 μM in 20% DMSO. An aliquot (10 μL) of each dilution was transferred to a 96-well microtiter plate, and freshly prepared microbial broth (190 μL) was added to each well to give final concentrations of 30−0.01 μM in 1% DMSO. The plates were incubated at 37 °C for 24 h, and the optical density of each well was measured spectrophotometrically at 600 nm using a POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Each test compound was screened against the Gram-negative bacteria *Escherichia coli*

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ATCC11775 and *Pseudomonas aeruginosa* ATCC10145; the Gram-positive bacteria *Staphylococcus aureus* ATCC25923, *B. subtilis* ATCC6633, and *Streptococcus pyogenes* (ATCC2727); and three clinical isolates *S. pyogenes* (D3840), vancomycin-resistant Enterococci (VRE), and methicillin-resistant *S. aureus* (MRSA). Rifampicin/ampicillin and vancomycin/methicillin were used as positive controls (40 μg/mL in 10% DMSO) for susceptible and multidrug-resistant strains, respectively. The IC_{50} value was calculated as the concentration of the compound or antibiotic required for 50% inhibition of the bacterial cells using Prism 7.0 (GraphPad Software Inc., La Jolla, CA). The outcomes of the relevant tests are shown in Figure S12.

Antifungal Assays. The fungus *C. albicans* ATCC10231 was streaked onto a Sabouraud agar plate and was incubated at 37 °C for 48 h. One colony was then transferred to fresh Sabouraud broth (15 mL) and the cell density adjusted to 10^4−10^5 CFU/mL. For the single data point experiments, an aliquot (10 μL) from 600 μM stock solution (20% DMSO) was transferred to a 96-well microtiter plate, and freshly prepared fungal broth (190 μL) was added to each well to give final concentrations of 30 μM in 1% DMSO. The plates were incubated at 37 °C for 24 h, and the optical density of each well was measured spectrophotometrically at 600 nm using a POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Ketoconazole was used as a positive control (30 μg/mL in 10% DMSO). Where relevant, IC_{50} values were calculated as the concentration of the compound or antifungal drug required for 50% inhibition of the fungal cells using Prism 7.0 (GraphPad Software Inc., La Jolla, CA).

Cytotoxicity Assays. Adherent human hepatocellular (HepG2), human colorectal (SW620), and lung carcinoma (NCI-H460) cells were cultured in Roswell Park Memorial Institute (RPMI) medium 1640. All cells were cultured as adherent monolayers in flasks supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin (100 unit/mL), and streptomycin (100 μg/mL) in a humidified 37 °C incubator supplied with 5% CO_2. Briefly, cells were harvested with trypsin and dispensed into 96-well microtiter assay plates at 3,000 cells/well after which they were incubated for 18 h at 37 °C with 5% CO_2 (to allow cells to attach as adherent monolayers). Test compounds were dissolved in DMSO and diluted with phosphate-buffered saline (PBS) to give a 600 μM stock solution (20% DMSO), which was serially diluted with 20% DMSO to give concentrations from 600 to 0.2 μM in 20% DMSO. An aliquot (10 μL) of each dilution was applied to cells to give final concentrations of 30−0.01 μM in 1% DMSO. After 48 h incubation at 37 °C with 5% CO_2, an aliquot (20 μL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS (5 mg/mL) was added to each well (final concentration of 0.5 mg/mL) and microtiter plates were incubated for further 4 h at 37 °C with 5% CO_2. After final incubation, the medium was aspirated and precipitated formazan crystals dissolved in DMSO (100 μL/well). The absorbance of each well was measured at 600 nm with a PowerWave XS Microplate Reader from Bio-Tek Instruments Inc. (Vinooski, VT). IC_{50} values were calculated using Prism 7.0 (GraphPad Software Inc., La Jolla, CA) as the concentration of the analyte required for 50% inhibition of cancer cell growth (compared to negative controls). The negative control was 1% aqueous DMSO, while the positive control was vinblastine (30 μM). All experiments were performed in duplicate. The outcomes of the relevant tests are shown in Figure S13.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01331.

Plots from crystallographic studies, additional biological test results, and ^{1}H and ^{13}C NMR spectra of compounds **9**−**19** and **21**−**35** (PDF)
X-ray data for compound **10** (CIF)
X-ray data for compound **13** (CIF)
X-ray data for compound **14** (CIF)
X-ray data for compound **16** (CIF)
X-ray data for compound **17** (CIF)
X-ray data for compound **22** (CIF)
X-ray data for compound **23** (CIF)
X-ray data for compound **29** (CIF)
X-ray data for compound **30** (CIF)
X-ray data for compound **32** (CIF)
X-ray data for compound **33** (CIF)

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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After this paper was published ASAP June 8, 2020, a correction was made to Scheme 1. The corrected version was reposted June 16, 2020.

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