Total Syntheses of the Amaryllidaceae Alkaloids Zephycandidine III and Lycosinine A and Their Evaluation as Inhibitors of Acetylcholinesterase

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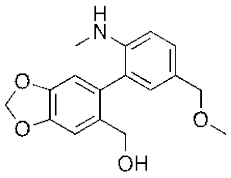
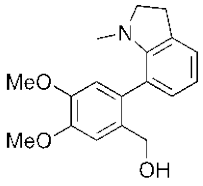


zephycandidine III
(**1**)

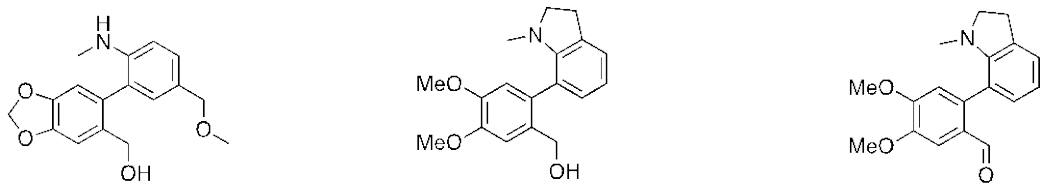


lycosinine A
(**2**)

The title alkaloids, **1** and **2** respectively, have been prepared using cross-coupling chemistries and together with various analogues they have been evaluated for their capacity to inhibit acetylcholinesterase. Contrary to an earlier report, we have found that biaryl **1** is not a significant inhibitor of this enzyme and nor are any of its congeners, including alkaloid **2**.

INTRODUCTION

Very recently Yao and co-workers reported[1] the isolation of three new Amaryllidaceae alkaloids through extraction of the dried whole plant *Zephyranhes candida* collected at Shiyan, Hubei in China. Their structures were established by conventional spectroscopic methods and each was evaluated as a possible inhibitor of acetylcholinesterase (AChE). The most active of these [with an IC_{50} of 8.82 μM] was reported to be the novel biaryl zephycandidine III (**1**) (Figure 1). This compound bears a strong structural resemblance to the Amaryllidaceae alkaloid lycosinine A (**2**) that was isolated by Zhao and co-workers[2] from the ornamental plant *Lycoris aurea* collected in Kunming, Yunnan Province, China. The corresponding aldehyde, *viz*. lycosinine B (**3**), was also obtained from the same source. Lycosinine B (**3**) has since been isolated from the bulbs of *Lycoris sprengeri* collected from Taizhou City, Zhejiang Province, China[3] and from the bulbs of *Hippeastrum breviflorum* Herb. Amaryllidaceae (flowering sage) collected in São Francisco de Paula in the Brazilian state of Rio Grande do Sul.[4] No biological activities have been ascribed to the lycosinines thus far but given the structural resemblance of compound **2** to zephycandidine III it might also be expected to display AChE-inhibiting activities.



1 2 3

Figure 1: Structures of the Amaryllidaceae Alkaloids Zephycandidine III (**1**), Lycosinine A (**2**) and Lycosinine B (**3**)

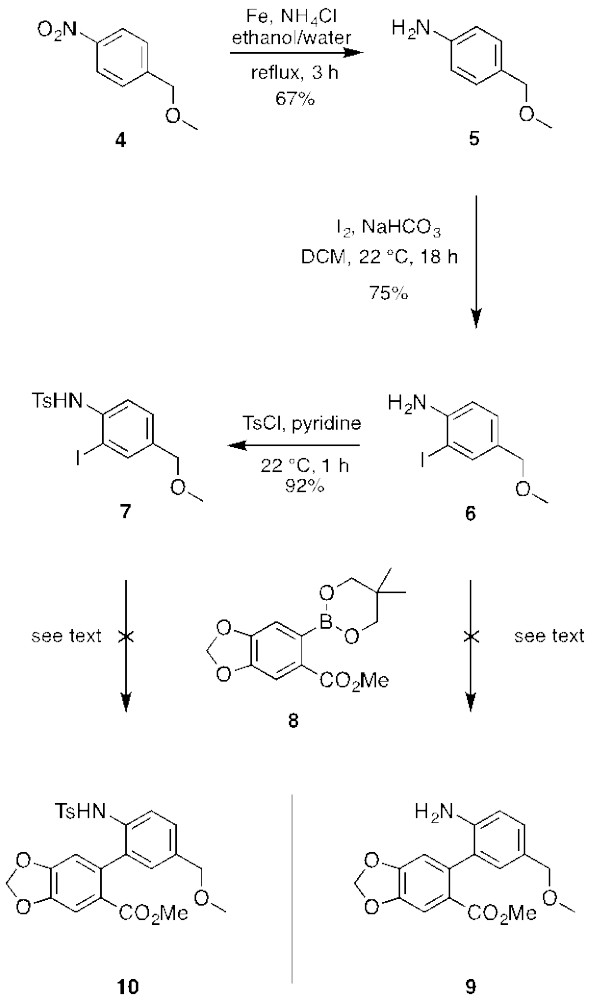
To date there have been no reports concerned with synthesis of zephycandidine III (**1**) and just one dealing with the preparation of the lycosinines. Specifically, Hsieh and co-workers reported[5] generating compounds **2** and **3** using, as the key step, the Suzuki-Miyuara cross-coupling of a borylated derivative of 3,4-dimethoxybenzaldehyde with 7-bromo-1-methylindoline. It is against this background that we now report our own studies in the area that have culminated in syntheses of the title alkaloids (*viz*. **1** and **2**) and thus allowed for their evaluation as inhibitors of AChE derived from *Electrophorus electricus*.

RESULTS AND DISCUSSION

1. Total Synthesis of Zephycandidine III (1)

Our initial approach to biaryl core of zephycandidine III (**1**) is shown in Scheme 1 and sought to exploit Suzuki-Miyuara cross-coupling chemistry. Thus, the known nitroarene **4**[6] was reduced to the corresponding and previously reported aniline **5**[7] (67%) using iron filings in mildly acidic aqueous ethanol and the later compound subjected to electrophilic aromatic iodination with molecular iodine.

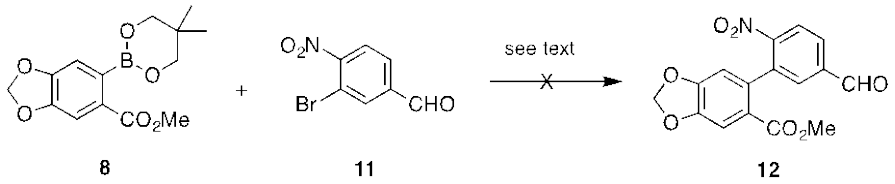
Scheme 1: Initial Attempts to Assemble the Biaryl Core of Zephycandidine III (**1**) Using Suzuki-Miyaura Cross-coupling Protocols



Product **6** (75%) was then converted into the corresponding *p*-toluenesulfonamide **7** (92%) under standard conditions. Disappointingly, and despite extensive experimentation involving a range of reaction conditions, attempts to engage aniline **6** in a Suzuki-Miyaura cross-coupling reaction with the known and readily accessible arylboronate **8**[8] failed to deliver the biaryl **9**. Related attempts to cross-couple sulfonamide **7** with ester **8** and thereby generate compound **10** were equally unsuccessful.

In a second approach (Scheme 2) to the biaryl core of target **1** that sought to exploit the often-beneficial effects of electron-withdrawing groups in Suzuki-Miyaura cross-coupling reactions,[9] attempts were made to link the boronate ester **8** and 3-bromo-4-nitrobenzaldehyde (**11**)[10] under a range of seemingly relevant reaction conditions. Unfortunately, none of these led to the formation of the hoped-for biaryl **12**.

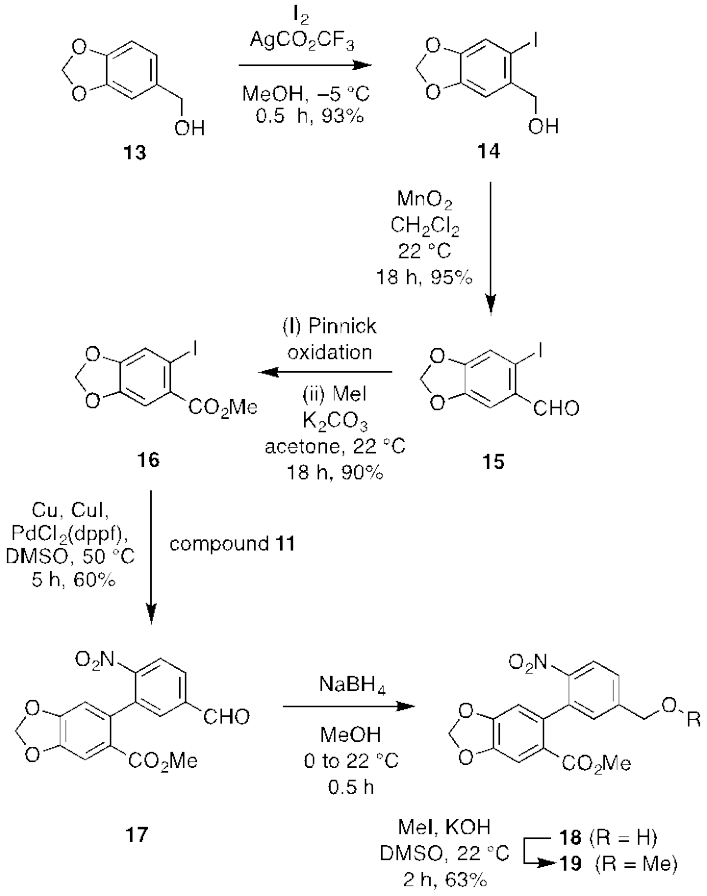
Scheme 2: An Alternate Attempt to Effect a Relevant Suzuki-Miyaura Cross-coupling Reaction



The palladium-catalyzed Ullmann cross-coupling reaction has proven to be a useful but under-utilized method for cross-coupling various aryl halides with other species, most notably α-iodoenones.[11] Given our familiarity with this process we sought to apply it in the present context. To such ends (Scheme 3) the commercially available alcohol **13** was treated with a combination of molecular iodine and silver trifluoroacetate and thereby

affording compound **14**[12] (93%) that was oxidized to corresponding aldehyde **15**[12] (95%) using Attenborrow manganese dioxide[13] under sonication conditions. Pinnick oxidation of compound **15** to the corresponding acid and esterification of this using methyl iodide in the presence of potassium carbonate then gave ester **16**[12] in 85% overall yield.

Scheme 3: Successful Synthesis of the Biaryl Core of Target **1**



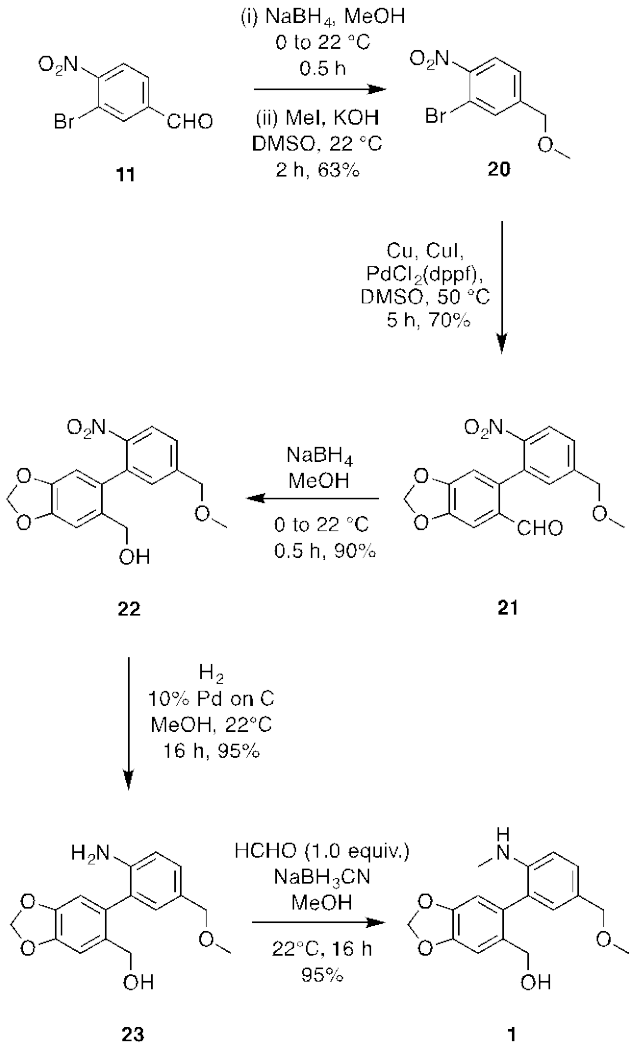
Gratifyingly, when a DMSO solution of this last compound was reacted with bromoarene **11** in the presence of copper metal as well as small amounts of CuI and $PdCl_2$(dppf) at 50 °C for 5 h then the biaryl **17** was obtained in 60% yield. Treatment of compound **17** with sodium borohydride in methanol gave the benzyl alcohol **18** and *O*-methylation of this

using methyl iodide in DMSO containing potassium hydroxide then afforded the methyl ether **19** (63%). The structure of compound **18** was confirmed by single-crystal X-ray analysis [see Experimental Section and Supporting Information (SI) for details]. Unfortunately, all attempts to effect the reduction of the nitro- and ester-groups associated with this last compound, and thereby generate zephycandidine III (**1**) directly, were unsuccessful. In every instance complex product mixtures were obtained.

The ultimately successful route to zephycandidine III (**1**) is displayed in Scheme 4 and exploited various of the chemistries defined in Scheme 3. Thus, the reaction sequence started with the conversion of aldehyde **11**, through a reduction/*O*-methylation sequence, into the methyl ether **20** (63%). Like its precursor **11**, compound **20** could be engaged in a palladium-catalyzed Ullmann cross-coupling, this time with aldehyde **15**, and thereby affording the biaryl **21**, the structure of which was confirmed by single-crystal X-ray analysis. The aldehydic residue within compound **21** was reduced with sodium borohydride and the nitro-group associated with product **22** (90%) subjected to hydrogenolysis using dihydrogen in the presence of 10% palladium on carbon. By such means aniline **22** was obtained in 95% yield. Reductive mono-methylation of compound **22** using one molar equivalent of formaldehyde in the presence of sodium cyanoborohydride then gave compound **1** as a clear, colorless oil in 95% yield. All of the spectral data acquired on this product were in complete according with the assigned structure. Furthermore, relevant comparisons with the analogous data reported by Yao and co-workers[1] for zephycandidine III revealed a good match (tabular comparisons of

the ^{13}C NMR data sets are provided in the SI) and thus leaving no doubt about the structure of the natural product.

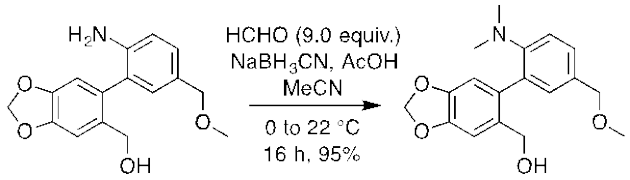
Scheme 4: Successful Total Synthesis of Zephycandidine III (**1**).



The two-fold or Eschweiler-Clark-type *N*-methylation of aniline **23** was readily effected (Scheme 5) using 9 molar equivalents of formaldehyde in the presence of sodium cyanaoborohydride and compound **24** was thereby obtained in 95% yield. This product is

the *N*-methyl derivative of zephycandidine III (**1**) and arguably, therefore, more closely resembles lycosinine A (**2**).

Scheme 5: Eschweiler-Clark Methylation of Aniline **23** Leading to the *N*-Methyl Derivative, **24**, of Zephycandidine III (**1**).



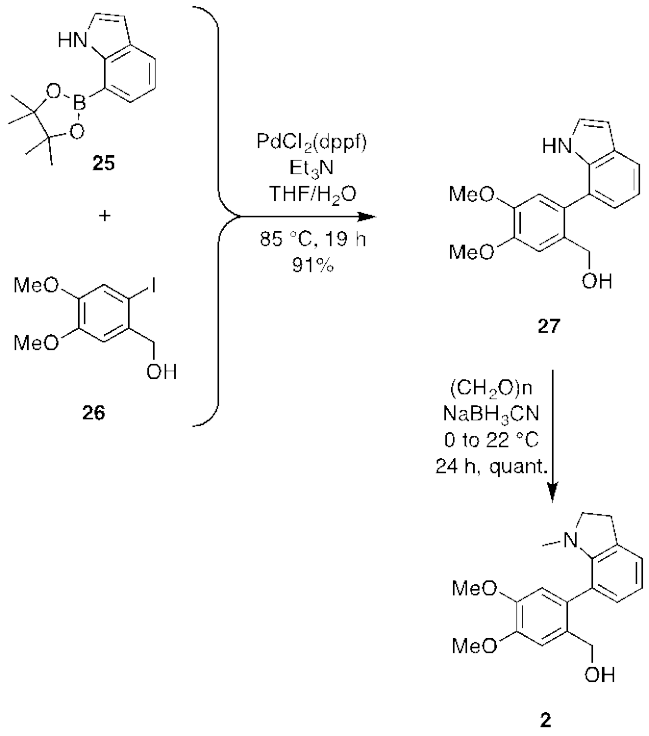
23 **24**

2. Total Synthesis of Lycosinine A (2) and an Examination of Its Behavior Under Oxidative Conditions

The two-step synthesis of lycosinine A (**2**) from known materials is shown in Scheme 6. This simply involved Suzuki-Miyaura cross-coupling of the known C7-borylated indole **25** (prepared in a one-pot process and 86% yield from indole itself using a procedure reported by Hartwig[14]) with the readily available aryl iodide **26**[15] under relatively standard conditions and so affording the 7-arylindole **27** (91%), the structure of which was confirmed by single-crystal X-ray analysis. Reductive *N*-methylation of the last compound using paraformaldehyde in the presence of sodium cyanoborohydride was accompanied by conversion of the indole residue into the corresponding indoline and so affording lycosinine A (**2**) directly and in 91% yield. All of the of spectral data acquired on this compound were in accord with the assigned structure and matched those reported[2] for the natural product (tabular comparisons of the ^{13}C NMR data sets are provided in the

SI). In addition, a single-crystal X-ray analysis of the synthetically derived material was carried out and served to confirm the illustrated structure.

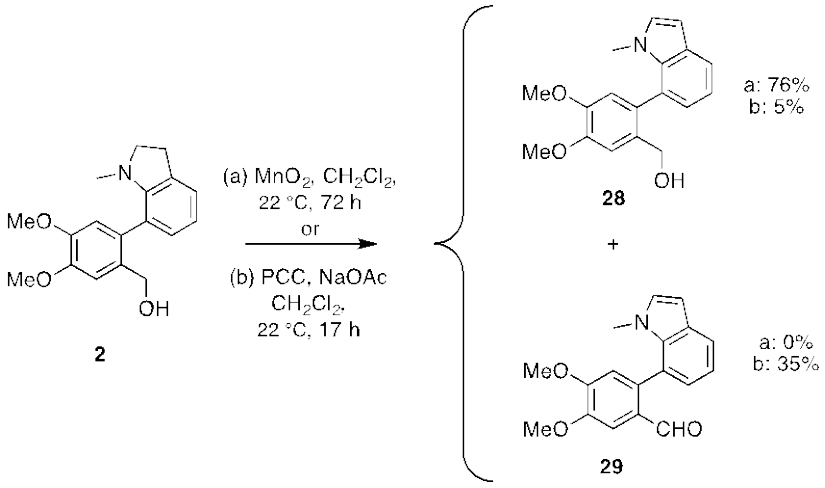
Scheme 6: Two-step Synthesis of Lycosinine A (**2**).



With compound **2** in hand various efforts were made to convert it into lycosinine B (**3**). However, despite numerous efforts this conversion failed due to the oxidative sensitivity of the indoline unit associated with the substrate. For example, on treating lycosinine A (**2**) (Scheme 7) with Attenborrow manganese dioxide[13] in dichloromethane indole **27** (76%) was obtained. On the other hand, when the same substrate (viz. **2**) was treated with pyridinium chlorochromate (PCC) in the presence of sodium acetate then indole **27** (5%) was again obtained but the two-fold oxidation product **28** (35%) was now the predominant one. It is interesting to note that in Hsieh's synthesis of lycosinine B (**3**)[5] the

associated aldehyde residue was installed directly through a cross-coupling reaction involving a C2-borylated benzaldehyde and this natural product was then reduced to lycosinine A (**2**).

Scheme 7: Outcomes of the Oxidation of Lycosinine A (**2**).



3. Evaluation of Compounds 1, 2, 17-19, 21-24 and 27 as Inhibitors of AChE

Alkaloids **1** and **2**, as well as congeners **17**-**19**, **21**-**24** and **27** were each evaluated for their ability to inhibit AChE derived from *Electrophorus electricus*.[16] A summary of the inhibition data thus obtained is shown in Table 1. Compounds **17** and **24** (viz. the *N*-methyl derivative of zephycandidine III) were the most effective inhibitors exhibiting IC_{50} values of 120 and 140 μM respectively, however this is two orders of magnitude weaker than the alkaloid galanthamine, an established AChE inhibitor used to treat Alzheimer's disease. The assay results are also at odds with those of Yao and co-workers who have suggested[1] that zephycandidine III (compound **1**) is a notable inhibitor of the

enzyme. When assayed against AChE, **1** reduced activity by only 25% at the maximum concentration tested (500 μM). The origins of the discrepancies between the work of Yao and co-workers and our own reported herein are unclear but could be the result of the natural product being contaminated with a potent but as yet unidentified inhibitor of AChE.

Table 1: Inhibition of AChE by compounds **1**, **2**, **17-19, 21-24** and **27**

Entry	Compound	IC_{50} (μM)[a]
1	**1**	>500[b]
2	**2**	>500
3	**17**	120 [110-150]
4	**18**	210 [180-260]
5	**19**	270 [190-430]
6	**21**	280 [250-330]
7	**22**	>500
8	**23**	>200
9	**24**	140 [130-160]
10	**27**	>500
14	Galanthamine (+ve control)	1.1 [0.9-1.2]

[a] Values in brackets represent the 95% confidence interval in the IC_{50}. The IC_{50} was calculated from a dose-response curve with three repeat measurements of enzyme activity at each concentration of compound. [b] A lower limit is given for compounds where the IC_{50} was greater than the maximum soluble concentration of the compound.

CONCLUSIONS

The studies reported herein serve to confirm the structures assigned to the title natural products but cast serious doubt on the merits of trying to develop related polyfunctionalized biaryls as new, effective inhibitors of AChE.

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise specified, proton (^1H) and carbon (^{13}C) NMR spectra were recorded at room temperature in base-filtered $CDCl_3$ on a Varian spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei.

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 77.0 were used to reference ^1H and ^{13}C NMR spectra, respectively. ^1H 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. Infrared spectra (ν_{max}) were recorded on a Perkin–Elmer 1800 Series FTIR Spectrometer. Samples were analyzed as thin films on KBr plates. 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 an Optimelt automated melting point system 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 (conc.) : 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.[17] with silica gel 60 (40–63 [illegible] or HPLC grade solvents indicated. The melting points of solids purified by such means were recorded directly (ie after they had crystallized from the concentrated chromatographic fractions). 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.[18] Where necessary, reactions were performed under an nitrogen atmosphere.

4-(Methoxymethyl)aniline (5). A magnetically stirred suspension of iron filings (5.41 g, 97.0 g.atom) in ethanol (30 mL) containing in NH_4Cl (12 ml of a 22% w/v aqueous solution) was treated with nitroarene **4**[6] (1.67 g, 10 mmol) and the ensuing mixture heated under reflux for 2 h then cooled and filtered. The filtrate was extracted with ethyl acetate (3 × 30 mL) and the combined organic phases washed with brine (1 × 50 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica gel, 5:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.3 in 5:1 v/v hexane/ethyl acetate) afforded compound **5**[7] (920 mg, 67%) as clear, light brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 4.33 (s, 2H), 3.66 (broad s, 2H), 3.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.2, 129.6, 128.2, 115.1, 74.8, 57.8.

2-Iodo-4-(methoxymethyl)aniline (6). A magnetically stirred solution of compound **5** (686 mg, 5.0 mmol) in CH_2Cl_2 (20 mL) maintained at 22 °C was treated with water (10 mL), $NaHCO_3$ (1.26 g, 15 mmol) and molecular iodine (1.27 g, 5.0 mmol). The ensuing mixture was stirred at 22 °C for a further 16 h then quenched with $Na_2S_2O_3$ (10 mL of a saturated aqueous solution) and separated aqueous layer extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine (1 × 25 mL) then dried

(Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing light-brown oil was subjected flash chromatography (silica gel, 10:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.4 in 5:1 v/v hexane/ethyl acetate) gave compound **6** (980 mg, 75%) as a clear, brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 1.9 Hz, 1H), 7.11 (dd, *J* = 8.1 and 1.9 Hz, 1H), 6.72 (d, *J* = 8.1 Hz, 1H), 4.29 (s, 2H), 4.09 (s, 2H), 3.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 138.9, 129.9, 129.5, 114.6, 84.0, 73.8, 57.9; IR (KBr) ν_{max} 3455, 3348, 2817, 1614, 1500, 1352, 1305, 1352, 1201, 1088, 1031, 885, 820, 666 cm^{-1}; MS (ESI, +ve) *m/z* 286 [$(M+Na)^+$, 100%]: HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_8H_{10}{}^{127}INNaO$ 285.9705, found 285.9705.

***N*-(2-Iodo-4-(methoxymethyl)phenyl)-4-methylbenzenesulfonamide (7)**. A solution of compound **6** (522 mg, 2.1 mmol) and *p*-toluenesulfonyl chloride (477 mg, 2.5 mmol) in pyridine (10 mL) was stirred at 22 °C for 4 h then water (30 mL) was added and the separated aqueous phase extracted with ethyl acetate (3 × 30 mL). The combined organic fractions were washed with $CuSO_4$ (1 × 50 ml of a 10% w/v aqueous solution) and water (1 × 50 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the ensuing light-yellow oil subjected to flash chromatography (silica gel, 5:1 v/v 40−60 petroleum spirit/ethyl acetate elution). Concentration of the relevant fractions (R_f = 0.3 in 3:1 v/v hexane/ethyl acetate) then gave compound **7** (806 mg, 92%) as white, crystalline solid, mp = 121.5-123 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.22 (dd, *J* = 8.2 and 1.8 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 4.32 (s, 2H), 3.26 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 143.1, 138.7, 138.3, 137.8, 137.3, 129.6, 127.9, 126.8, 98.6, 71.9, 57.7, 21.0 (one signal obscured or overlapping); IR (KBr) ν_{max} 3289,1598, 1489, 1386, 1335, 1164, 1091, 1037,916, 814, 667, 545 cm^{-1}; MS (ESI, +ve) *m/z* 440 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{15}H_{16}{}^{127}INNaO_3S$ 439.9793, found 439.9794.

3-Bromo-4-nitrobenzaldehyde (11). Compound **11** was prepared according to the method of Katritzky[10] and obtained as crystalline yellow solid, mp = 99-101 °C (lit.[10] mp. = 101-102 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.23 (d, *J* = 1.6 Hz, 1H), 7.97 (dd, *J* = 8.2 and 1.6 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 153.2, 139.0, 136.0, 129.1, 126.1, 115.4; IR (KBr) ν_{max} 3094, 1694, 1584, 1529, 1373, 1360, 1300, 1191, 1037, 900, 841, 831, 748, 699, 682 cm^{-1}; MS (EI, 70 eV) *m/z* 231 and 229 ($M^{+\bullet}$, 88 and 90%), 75 (100); HRMS (EI, 70 eV) $M^{+\bullet}$ calcd for $C_7H_4{}^{79}BrNO_3$ 228.9375, found 228.9376.

(6-Iodobenzo[*d*][1,3]dioxol-5-yl)methanol (14). A flame-dried 200 mL round-bottom flask was covered with aluminium foil then charged with piperonyl alcohol (3.00 g, 19.7 mmol), $AgOCOCF_3$ (4.80 g, 22.0 mmol), a magnetic stirring bar and $CHCl_3$ (20 mL). The ensuing mixture was cooled to −5 °C (ice-salt bath) then treated, dropwise over ca. 0.10 h, with a solution of molecular iodine (5.50 g, 18.0 mmol) in $CHCl_3$ (25 mL). Additional quantities of solid molecular iodine were added to the reaction mixture until TLC analysis revealed that all of the starting alcohol had been consumed. The reaction mixture was then filtered through a pad of tightly packed diatomaceous earth and the solids thus retained rinsed with $CHCl_3$ (150 mL). The combined filtrates were washed with $Na_2S_2O_3$ (2 × 200 mL of a 2 M aqueous solution) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting solid was subjected to

flash chromatography (silica gel, 5:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.2 in 4:1 v/v 40−60 petroleum spirit/ethyl acetate) afforded compound **14**[12] (5.10 g, 93%) as a white, crystalline solid, mp = 109-111 °C (lit.[12a] mp. = 109.3-109.8 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (s, 1H), 6.99 (s, 1H), 5.98 (s, 2H), 4.59 (s, 2H), 1.88 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 148.1, 136.4, 118.7, 109.2, 101.8, 85.5, 69.4.

6-Iodobenzo[*d*][1,3]dioxole-5-carbaldehyde (15). A magnetically stirred solution of compound **14** (4.00 g, 14.4 mmol) in CH_2Cl_2 (75 mL) was treated, in one portion, with MnO_2 (12.5 g, 143.9 mmol) and the resulting dark suspension was stirred at 22 °C for 18 h then filtered through pad of diatomaceous earth. The solids thus retained were washed with CH_2Cl_2 (2 × 80 mL) and the combined filtrates were concentrated under reduced pressure. The resulting brown oil was subjected to flash chromatography (silica, 4:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.4 in 4:1 v/v 40−60 petroleum spirit/ethyl acetate) then gave aldehyde **15**[12] (3.85 g, 97%) as a white, crystalline solid, mp = 112-113 °C (lit.[12a] mp. = 112-113 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.37 (s, 1H), 7.34 (s, 1H), 6.08 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.6, 153.7, 149.3, 129.8, 119.6, 109.0, 102.8, 93.5; IR (KBr) ν_{max} 1664, 1610, 1505, 1386, 1268, 1113, 925 cm^{-1}; MS (ESI, +ve) *m/z* 331 (100%), 299 [$(M+Na)^+$, 45]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_8H_5{}^{127}INaO_3$ 298.9181, found 298.9188.

Methyl 6-Iodobenzo[*d*][1,3]dioxole-5-carboxylate (16). *Step i*: A magnetically stirred solution of aldehyde **15** (3.60 g, 13.0 mmol) and 2-methyl-2-butene (7.6 mL, 65.0 mmol) in acetone (50 mL) maintained at 0 °C (ice bath) was treated, dropwise, with a solution of

sodium chlorite (4.40 g of 80% technical grade material, 39.0 mmol) and sodium dihydrogen phosphate dihydrate (6.10 g, 39.0 mmol) in water (20 mL). The ensuing mixture was allowed to warm to 22 °C, stirred at this temperature for 3 h then treated with NH_4Cl (60 mL of a saturated aqueous solution). The resulting mixture was extracted with ethyl acetate (3 × 100 mL) and the combined organic phases were washed with brine (1 × 150 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the anticipated acid (3.42 g, 90%) as white, crystalline solid. The material was used without purification in next step of the reaction.

Step ii: A magnetically stirred solution of the acid (3.42 g, 11.7 mmol) from step i in acetone (60 mL) was treated with potassium carbonate (4.85 g, 35.1 mmol). The ensuing mixture was stirred at 22 °C for 0.25 h before treated with iodomethane (2.5 mL, 58.5 mmol) then stirred at 22 °C for 18 h before being passed through a pad of TLC-grade silica gel. The filtrate thus obtained was concentrated under reduced pressure to give ester **16**[12] (3.22 g, 90%) as a white, crystalline solid, mp = 83-85 °C (lit.[12a] mp = 84.6-86.1 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.37 (s, 1H), 6.04 (s, 2H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 151.2, 148.3, 127.7, 121.1, 111.2, 102.5, 85.0, 52.5; IR (KBr) ν_{max} 2951, 2905, 1727, 1614, 1502, 1480, 1435, 1378, 1348, 1243, 1133, 1088, 1037, 934, 776 cm^{-1}; MS (EI, 70 eV) *m/z* 306 ($M^{+\bullet}$, 100%], 275 (72); HRMS (EI, 70 eV) $M^{+\bullet}$ calcd for $C_9H_7{}^{127}IO_4$ 305.9389, found 305.9390.

Methyl 6-(5-Formyl-2-nitrophenyl)benzo[*d*][1,3]dioxole-5-carboxylate (17). A magnetically stirred mixture of compound **11** (510 mg, 2.2 mmol), ester **16** (814 mg, 2.6 mmol), copper powder (699 mg, 11 g.atom), CuI (628 mg, 3.3 mmol) and $Pd(dppf)Cl_2$ (161 mg, 0.22 mmol) in degassed DMSO (25 mL) was heated at 50 °C under a nitrogen

atmosphere for 12 h. The cooled reaction mixture was treated with water (30 mL) then diluted with ethyl acetate (40 mL) before being filtered through a pad of diatomaceous earth. The pad and the solids thus retained were washed with ethyl acetate (2 × 40 mL) and the separated organic phase associated with the filtrate was washed with water (2 × 50 mL) and brine (2 × 40 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a brown oil. Subjection of this material to flash chromatography (silica gel, 20:1 → 5:1 40−60 petroleum spirit/ethyl acetate elution) afforded, after concentration of the appropriate fractions (R_f = 0.2 in 5:1 v/v 40−60 petroleum spirit/ethyl acetate), compound **17** (427 mg, 59%) as a yellow crystalline solid, mp = 150.5–152.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.10 (s, 1H), 8.18 (d, *J* = 8.3 Hz, 1H), 8.01 (dd, *J* = 8.3 and 1.8 Hz, 1H), 7.78 (d, *J* = 1.8 Hz, 1H), 7.54 (s, 1H), 6.69 (s, 1H), 6.13 (ABq, *J* = 1.3 Hz, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.3, 165.7, 151.7, 151.2, 148.0, 138.4, 138.1, 134.4, 132.6, 129.2, 124.7, 122.0, 110.6, 110.2, 102.6, 52.2; IR (KBr) ν_{max} 2981, 2890, 1710, 1615, 1585, 1530, 1505, 1490, 1436, 1382, 1350, 1256, 1168, 1134, 1037, 930, 841 cm^{-1}; MS (ESI, +ve) *m/z* 384 [$(M+CH_3OH+Na)^+$, 100%], 352, [$(M+Na)^+$, 5], 330 (<1); HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{16}H_{11}NNaO_7$ 352.0433, found 352.0437.

Methyl 6-(5-(Hydroxymethyl)-2-nitrophenyl)benzo[*d*][1,3]dioxole-5-carboxylate (18). A suspension of $NaBH_4$ (23 mg, 0.61 mmol) in methanol (3.0 mL) was added to a magnetically stirred solution of compound **17** (200 mg, 0.61 mmol) in methanol (5 mL) maintained at 0 °C. The ensuing mixture was allowed to warm to 22 °C then stirred at this temperature for 0.5 h before being quenched with NaOH (5 mL of a 15% w/v aqueous solution). The ensuing mixture was stirred until it became homogeneous and

then it was concentrated under reduced pressure and the residue extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with $NaHCO_3$ (2 × 10 mL of a saturated aqueous solution) then H_2O (2 × 10 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a light yellow oil. Subjection of this material to flash chromatography (silica gel, 5:1 → 1:1 v/v 40-60 petroleum ether/ ethyl acetate elution) gave, after concentration of the appropriate fractions (R_f = 0.25 in 2:1 v/v petroleum ether/ethyl acetate) a yellow solid. Recrystallization ($CHCl_3$/hexane) of this solid then gave compound **18** (182 mg, 90%) as yellow crystals, mp = 114.5-116.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.47 (dd, *J* = 8.4 and 1.8 Hz, 1H), 7.24 (d, *J* = 1.8 Hz, 1H), 6.66 (s, 1H), 6.10 (s, 1H), 6.09 (s, 1H), 4.78 (s, 2H), 3.62 (s, 3H) (signal due to hydroxyl group proton not observed); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 151.0, 147.5, 147.4, 146.3, 137.5, 136.1, 129.2, 126.0, 124.5, 122.0, 110.4, 110.3, 102.4, 64.1, 52.1; IR (KBr) ν_{max} 3425, 2953, 2908, 1716, 1615, 1520, 1505, 1343, 1249, 1132, 1035, 928, 819 cm^{-1}; MS (ESI, +ve) *m/z* 354 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{16}H_{13}NNaO_7$ 354.0590, found 354.0588.

Methyl 6-(5-(Methoxymethyl)-2-nitrophenyl)benzo[*d*][1,3]dioxole-5-carboxylate (19). A solution of compound **18** (100 mg, 0.30 mmol) and methyl iodide (75 µL, 1.2 mmol) in DMSO (2 mL) was added, in portions over 1.5 h, to a magnetically stirred suspension of potassium hydroxide (68 mg, 1.2 mmol) in DMSO (3 mL) maintained at 22 °C under an atmosphere of nitrogen. The ensuing mixture was stirred for a further 1.0 h then poured into water (25 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with water (1 × 50 mL) and brine (1 × 50 mL) before being dried (Na_2SO_4), filtered and then concentrated under reduced pressure. The oily residue

thus obtained was subjected to flash chromatography (silica gel, 4:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.4 in 4:1 v/v hexane/ethyl acetate) afforded compound **19** (80 mg, 77%) as yellow, crystalline solid, mp = 150.7-153.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.44 (dd, *J* = 8.4 and 1.8 Hz, 1H), 7.21 (d, *J* = 1.8 Hz, 1H), 6.67 (s, 1H), 6.09 (s, 1H), 6.09 (s, 1H), 4.53 (s, 2H), 3.61 (s, 3H), 3.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 151.0, 147.5, 143.9, 137.5, 136.1, 129.9, 126.7, 124.3, 122.1, 110.4, 110.3, 102.4, 73.4, 58.8, 52.1 (signal due to one carbon obscured or overlapping); IR (KBr) ν_{max} 2903, 1720, 1615, 1522, 1505, 1490, 1480, 1436, 1374, 1346, 1249, 1132, 1096, 1037, 842, 766 cm^{-1}; MS (ESI, +ve) *m/z* 368 [(M+Na)$^+$, 100%]; HRMS (ESI, +ve) (M+Na)$^+$ calcd for $C_{17}H_{15}NNaO_7$ 368.0746, found 368.0741.

2-Bromo-4-(methoxymethyl)-1-nitrobenzene (20). *Step i*: A suspension of $NaBH_4$ (165 mg, 4.36 mmol) in methanol (15 mL) was added in portions over 0.17 h to a magnetically stirred solution of compound **11** (1.50 g, 6.50 mmol) in methanol (30 mL) maintained at 22 °C. The ensuing mixture was stirred for a further 0.5 h then quenched with NaOH (40 mL of a 10% w/v aqueous solution). Stirring was continued until a clear solution was obtained and this was then concentrated under reduced pressure and the aqueous residue extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with $NaHCO_3$ (2 × 50 mL of saturated aqueous solution) and water (2 × 30 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a yellow solid presumed to be (3-bromo-4-nitrophenyl)methanol. This material was used without purification in *Step ii* as detailed immediately below.

Step ii: A solution of the product from step i and methyl iodide (1.60 mL, 26 mmol) in DMSO (10 mL) was added over 1.5 h to a magnetically stirred suspension of potassium hydroxide (1.46 g, 26 mmol) in DMSO maintained at 22 °C. The resulting mixture was stirred for a further 1 h then poured into water (150 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were washed with water (1 × 150 mL) and brine (1 × 50 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica gel, 4:1 v/v 40−60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (R_f = 0.5 in 4:1 v/v 40−60 petroleum spirit/ethyl acetate) afforded compound **20** (1.15 g, 72%) as a clear, light-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.3 Hz, 1H), 7.72 (d, *J* = 0.8 Hz, 1H), 7.40 (dd, *J* = 8.3 and 0.8 Hz, 1H), 4.50 (s, 2H), 3.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 133.3, 126.5, 125.7, 114.7, 100.0, 72.6, 58.7; IR (KBr) ν_{max} 2932, 2823, 1584, 1528, 1469, 1347, 1197, 1109, 1039, 825, 747 cm^{-1}; MS (ESI, +ve) *m/z* 270 and 268 [$(M+Na)^+$, both 10%], 248 and 246 (17 and 20), 87 (100); HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_8H_8{}^{79}BrNNaO_3$ 267.9585, found 267.9581.

6-(5-(Methoxymethyl)-2-nitrophenyl)benzo[*d*][1,3]dioxole-5-carbaldehyde (21). A magnetically stirred mixture of compound **20** (246 mg, 1 mmol), aryl iodide **15** (359 mg, 1.3 mmol), copper powder (318 mg, 5 mmol), CuI (286 mg, 1.5 mmol) and Pd(dppf)Cl_2 (73 mg, 0.1 mmol) in degassed DMSO (10 mL) was heated at 50 °C under a nitrogen atmosphere for 12 h. The cooled reaction mixture was quenched with water (20 mL) then diluted with ethyl acetate (30 mL) before being filtered through a pad of diatomaceous earth. The pad and the solids thus retained were washed with ethyl acetate (2 × 30 mL) and the combined organic phases were washed with water (2 × 40 mL) and brine (2 × 40

mL) before being dried (Na_2SO_4), filtrated and concentrated under reduced pressure to give a light brown oil. Subjection of this material to flash chromatography (silica gel, 15:1 → 3:1 v/v 40-60 petroleum ether/ ethyl acetate gradient elution) gave, after concentration of the appropriate fractions (R_f = 0.2 in 5:1 v/v 40-60 petroleum ether/ ethyl acetate elution), compound **21** (190 mg, 70%) as a light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.53 (dd, *J* = 8.4 and 1.8 Hz, 1H), 7.44 (s, 1H), 7.33 (d, *J* = 1.8 Hz, 1H), 6.67 (s, 1H), 6.11 (ABq, *J* = 1.1 Hz, 2H), 4.55 (s, 2H), 3.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.0, 152.3, 148.6, 148.2, 144.2, 138.1, 133.3, 131.2, 129.0, 127.7, 124.8, 109.7, 107.5, 102.6, 73.2, 58.9; IR (KBr) ν_{max} 2922, 1681, 1611, 1522, 1477, 1412, 1345, 1261, 1238, 1136, 1107, 1036, 931, 880, 844, 792, 764 cm^{-1}; MS (ESI, +ve) *m/z* 338 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{16}H_{13}NNaO_6$ 338.0641, found 338.0638.

(6-(5-(Methoxymethyl)-2-nitrophenyl)benzo[*d*][1,3]dioxol-5-yl)methanol (22). A suspension of $NaBH_4$ (18 mg, 0.48 mmol) in methanol (2 mL) was added, in portions over 0.17 h, to a magnetically stirred solution of compound **21** (150 mg, 0.48 mmol) in methanol (3 mL) maintained at 0 °C. The ensuing mixture was allowed to warm to 22 °C, stirred at this temperature for 0.5 h then quenched with a sodium hydroxide (5 mL of a 10% w/v aqueous solution). Stirring was continued until a clear solution had been obtained and this was then concentrated under reduced pressure. The resulting aqueous residue was extracted with CH_2Cl_2 (3 × 6 mL) and the combined organic phases washed with $NaHCO_3$ (2 × 10 mL of a saturated aqueous solution) then water (2 × 10 mL) before being dried (Na_2SO_4, filtered and concentrated under reduced pressure to give a light-yellow oil. Subjection of this material to flash chromatography (silica gel, 6:1 → 2:1

40−60 petroleum spirit/ethyl acetate gradient elution) gave, after concentration of the appropriate fractions (R_f = 0.4 in 2:1 v/v 40−60 petroleum spirit/ethyl acetate), compound **22** (137 mg, 90%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.4 Hz, 1H), 7.47 (dd, *J* = 8.4 and 1.8 Hz, 1H), 7.31 (d, *J* = 1.8 Hz, 1H), 7.01 (s, 1H), 6.58 (s, 1H), 6.01 (ABq, *J* = 1.4 Hz, 2H), 4.53 (s, 2H), 4.30 (ABq, *J* = 12.3 Hz, 2H), 3.44 (s, 3H) (signal due to hydroxyl group proton not observed); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 148.1, 147.2, 143.8, 135.2, 132.3, 131.0, 130.1, 127.2, 124.5, 109.1, 101.6, 73.4, 63.1, 58.9; IR (KBr) ν_{max} 3405, 2892, 1523, 1502, 1476, 1347, 1227, 1105, 1038, 931, 841 cm^{-1}; MS (ESI, +ve) *m/z* 340 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{16}H_{15}NNaO_6$ 340.0797, found 340.0796.

(6-(2-Amino-5-(methoxymethyl)phenyl)benzo[*d*][1,3]dioxol-5-yl)methanol (23). A magnetically stirred solution of compound **22** (125 mg, 0.40 mmol) in methanol (8 mL) containing 10% Pd on carbon (20 mg) was placed under a hydrogen atmosphere at 22 °C for 12 h then filtered through a short pad of diatomaceous earth that was washed with ethyl acetate (20 mL). The combined filtrates were concentrated under reduced pressure to afford a light yellow oil and this was subjected to flash chromatography (silica gel, 9:1 v/v CH_2Cl_2/diethyl ether). Concentration of the appropriate fractions (R_f = 0.3 in 9:1 v/v CH_2Cl_2/diethyl ether) then provided the title compound **23** (108 mg, 95%) as a clear, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 8.1 and 2.0 Hz, 1H), 7.01 (d, *J* = 2.0 Hz, 1H), 7.01 (s, 1H), 6.83 (d, *J* = 8.1 Hz, 1H), 6.69 (s, 1H), 5.99 (ABq, *J* = 1.4 Hz, 2H), 4.36 (s, 2H), 4.28 (ABq, *J* = 12.0 Hz, 2H), 3.45 (broad s, 3H), 3.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8, 147.6, 142.2, 133.7, 131.0, 130.5, 130.1, 128.9, 128.2, 116.7, 110.2, 110.1, 101.4, 74.6, 63.8, 58.1; IR (KBr) ν_{max} 3360, 2889, 1621, 1502, 1482,

1365, 1224, 1086, 1038, 931, 872, 826 cm^{-1}; MS (ESI, +ve) *m/z* 310 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{16}H_{17}NNaO_4$ 310.1055, found 310.1055.

(6-(5-(Methoxymethyl)-2-(methylamino)phenyl)benzo[*d*][1,3]dioxol-5-yl)methanol (Zephcandidine III, 1). A magnetically stirred solution of compound **23** (30 mg, 0.11 mmol) in methanol (10 mL) maintained at 0 °C was treated with formaldehyde (8 µL of a 37% aqueous solution, 0.11 mmol). The ensuing mixture stirred for 0.5 h at 0 °C then treated with $NaBH_3CN$ (7 mg, 0.11 mmol) before being warmed to 22 °C and stirred at this temperature for 6 h then concentrated under reduced pressure to afford a light yellow oil. This was partitioned between ethyl acetate (10 mL) and NaOH (12 mL of a 1 M aqueous solution) and the separated aqueous phase was extracted with ethyl acetate (1 × 10 mL). The combined organic extracts were washed with brine (1 × 20 mL) then dried (Na_2SO_4), filtered and concentrated under reduced pressure. The light-yellow oil thus obtained was subjected to flash chromatography (silica gel, 20:1 → 5:1 v/v CH_2Cl_2/diethyl ether gradient elution) and concentration of the relevant fractions (R_f = 0.3 in 9:1 v/v CH_2Cl_2/diethyl ether) afforded compound **1** (25 mg, 80%) as a clear, colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (dd, *J* = 8.3 and 2.0 Hz, 1H), 7.06 (s, 1H), 6.90 (d, *J* = 2.0 Hz, 1H), 6.67 (d, *J* = 8.3 Hz, 1H), 6.59 (s, 1H), 5.97 (s, 2H), 4.34 (s, 2H), 4.23 (ABq, *J* = 12.7 Hz, 2H), 3.33 (s, 2H), 2.73 (s, 3H) (signals due to hydroxyl and amino group protons not observed); ^{13}C NMR (100 MHz, CD_3OD) δ 149.1, 148.6, 148.4, 135.2, 131.9(0), 131.8(6), 130.5, 127.4, 127.1, 111.3, 110.9, 109.3, 102.6, 76.0, 62.7, 57.9, 31.0; IR (KBr) ν_{max} 3420, 2919, 2889, 2817, 1611, 1519, 1503, 1484, 1246, 1223, 1085, 1038, 933, 871, 814 cm^{-1}; MS (ESI, +ve) *m/z* 324 [$(M+Na)^+$, 100%]; HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{17}H_{19}NNaO_4$ 324.1212, found 324.1210.

(6-(2-(Dimethylamino)-5-(methoxymethyl)phenyl)benzo[*d*][1,3]dioxol-5-yl)methanol (24). A magnetically stirred solution of compound **23** (40 mg, 0.14 mmol) in acetonitrile (2.0 mL) maintained at 22 °C was treated with formaldehyde (110 μL of a 37% aqueous solution, 1.32 mmol), acetic acid (40 μL, 0.70 mmol) and $NaBH_3CN$ (46 mg, 0.73 mmol). The ensuing mixture was stirred for 1 h then quenched with $NaHCO_3$ (5 mL of a saturated aqueous solution) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with brine (1 × 20 mL) before being dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The ensuing light-yellow oil was subjected to flash column chromatography (silica gel, 15:1 → 5:1 v/v CH_2Cl_2/diethyl ether gradient elution) and concentration of the appropriate fractions (R_f = 0.4 in 9:1 v/v CH_2Cl_2/diethyl ether) afforded compound **24** (42 mg, 95%) as a clear, colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.28 (dd, *J* = 8.3 and 2.2 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 7.01 (s, 1H), 6.71 (s, 1H), 5.98 (s, 2H), 4.41 (s, 2H), 4.29 (d, *J* = 12.5 Hz, 1H), 4.18 (d, *J* = 12.5 Hz, 1H), 3.36 (s, 3H), 2.51 (s, 6H) (signal due to hydroxyl group proton not observed); ^{13}C NMR (100 MHz, CD_3OD) δ 152.3, 148.6, 148.5, 135.3, 134.7, 133.8, 133.1, 133.0, 129.4, 118.8, 111.1, 109.6, 102.5, 75.3, 63.7, 58.1, 43.7; IR (KBr) ν_{max} 3399, 2981, 2883, 2835, 1502, 1484, 1411, 1222, 1129, 1094, 1039, 933, 869, 822 cm^{-1}; MS (ESI, +ve) *m/z* 338 [$(M+Na)^+$, 100%], 298 (54); HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{18}H_{21}NNaO_4$ 338.1368, found 338.1372.

7-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole (25). A magnetically stirred solution of indole (502 mg, 4.29 mmol), $[Ru(p\text{-cymene})Cl_2]_2$ (52 mg, 0.09 mmol, 2 mol%) and diethylsilane (1.7 mL, 13.1 mmol) in degassed toluene (2 mL) was stirred at 90 °C for 13 h then cooled to 22 °C and concentrated under reduced pressure to give a

brown oil. $[Ir(OMe)COD]_2$ (43 mg, 0.065 mmol, 3 mol%), di-*tert*-butylbipyridine (35 mg, 0.13 mmol, 3 mol %), bis(pinacolato)diboron (1.09 g, 4.29 mmol), pinacolborane (0.09 mL, 0.62 mmol) and degassed THF (4 mL) were then added to this oil and the ensuing mixture stirred at 80 °C for 16 h. After cooling to 22 °C the volatile components of the reaction mixture were removed under reduced pressure at 40 °C and the resulting brown oil was dissolved in THF (4 mL). The solution thus obtained was treated with sodium acetate (2.1 mL of a 3 M aqueous solution) and the ensuing mixture stirred at 22 °C for 2 h before being diluted with diethyl ether (40 mL) and water (40 mL). The separated aqueous phase was extracted with diethyl ether (2 × 40 mL) and the combined organic phases were washed with brine (1 × 40 mL) before being dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford a brown oil. Subjection of this material to flash chromatography (silica, 5:95 v/v diethyl ether/pentane elution) and concentration of the appropriate fractions (R_f = 0.2) gave compound **25** (893 mg, 86%) as a white, crystalline solid, mp = 89-90 °C (lit.[13] mp = 83-85 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.25 (broad s, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.28 (m, 1H), 7.15 (m, 1H), 6.56 (m, 1H), 1.41 (s, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 140.9, 129.2, 126.8, 124.2, 124.0, 119.3, 101.9, 83.8, 25.0 (signal due to one carbon obscured or overlapping); IR ν_{max} (KBr) 3454, 3398, 3055, 2977, 2930, 1595, 1511, 1429, 1369, 1330, 1296, 1272, 1192, 1145, 1130, 973, 857, 842, 798, 733 cm^{-1}; MS (EI, 70 eV) *m/z* 243 and 242 ($M^{+\bullet}$, 39 and 100%), 228 (15), 187 (21), 186 and 185 (93 and 38), 170 (28), 161 (16), 158 (17), 144 (33), 143 and 142 (78 and 33), 117 (16), 116 (23); HRMS (EI, 70 ev) $M^{+\bullet}$ calcd for $C_{14}H_{18}{}^{11}BNO_2$ 243.1429, found 243.1431.

(2-(1*H*-Indol-7-yl)-4,5-dimethoxyphenyl)methanol (27). A magnetically stirred

solution of indole **25** (406 mg, 1.67 mmol), aryl iodide **26**[14] (327 mg, 1.11 mmol), $PdCl_2(dppf)•CH_2Cl_2$ (90 mg, 0.11 mmol) and triethylamine (0.78 mL, 5.59 mmol) in degassed THF/water (3 mL of a 4:1 v/v mixture) was stirred in a sealed vessel at 85 °C for 19 h then cooled to 22 °C before being diluted with ethyl acetate (40 mL) and water (40 mL). The separated aqueous phase was extracted with ethyl acetate (2 × 40 mL) and the combined organic phases were passed through a pad of TLC-grade silica gel and diatomaceous earth. The filtrate thus obtained was concentrated under reduced pressure to give a brown oil and subjection of this material to flash chromatography (silica, 7:3 v/v ethyl acetate/hexane elution) followed by concentration of the appropriate fractions (R_f = 0.4) gave a brown solid. Recrystallization (methanol) of this material afforded the title compound **27** (285 mg, 91%) as a white, crystalline solid, mp = 173-174 °C. ^{1}H NMR (CD_3OD, 800 MHz) δ 7.54 (d, *J* = 7.5 Hz, 1H), 7.25 (s, 1H), 7.17 (d, *J* = 2.9 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.90 (s, 1H), 6.49 (d, *J* = 2.9 Hz, 1H), 4.37 (m, 2H), 3.91 (s, 3H), 3.80 (s, 3H) (signals due to hydroxyl and indole N-H group protons not observed); ^{13}C NMR (CD_3OD, 200 MHz) δ 150.0, 149.5, 136.1, 133.3, 131.6, 129.7, 126.0, 125.4, 123.5, 120.5, 120.1, 114.8, 113.1, 102.7, 62.5, 56.5(2), 56.4(6); IR ν_{max} (KBr) 3363, 3053, 3000, 2935, 2846, 1607, 1519, 1507, 1463, 1427, 1346, 1335, 1244, 1208, 1142, 1077, 995, 803, 734 cm^{-1}; MS (EI, 70 eV) *m/z* 283 ($M^{+\bullet}$, 46%), 265 (54), 264 (100); HRMS (EI, 70 ev) $M^{+\bullet}$ calcd for $C_{17}H_{17}NO_3$ 283.1208, found 283.1208.

(4,5-Dimethoxy-2-(1-methylindolin-7-yl)phenyl)methanol (Lycosinine A, 2). A magnetically stirred solution of compound **27** (283 mg, 1.00 mmol) and paraformaldehyde (300 mg, 9.99 mmol) in glacial acetic acid (3 mL) was cooled to 0 °C then treated, in one portion, with sodium cyanoborohydride (314 mg, 5.00 mmol). The

ensuing mixture was warmed to 18 °C, stirred at this temperature for 24 h then diluted with water (10 mL) before being cooled to 0 °C then treated, dropwise, with NaOH (5 M aqueous solution) until pH 13 was attained. The resulting mixture was extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic phases were washed with brine (1 × 40 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a clear, colorless oil. Subjection of this material to flash chromatography (silica, 7:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.4) gave a clear, colorless oil. Trituration (hexane) of this material at 10 °C afforded the title compound **2**[2] (298 mg, quantitative) as a white, crystalline solid, mp = 104-107 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.15 (dm, *J* = 7.2 Hz, 1H), 7.00 (s, 1H), 6.94 (dm, *J* = 7.2 Hz, 1H), 6.90 (t, *J* = 7.2 Hz, 1H), 6.84 (s, 1H), 4.93, (broad s, 1H), 4.22 (s, 2H), 3.95 (s, 3H), 3.88 (s, 3H), 3.60 (m, 1H), 3.12-3.06 (complex m, 1H), 3.01-2.91 (complex m, 2H), 2.20 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.0, 148.5, 148.4, 132.1, 132.0, 131.7, 129.7, 126.2, 123.8, 120.9, 112.9, 112.5, 64.6, 56.6, 56.0, 55.9, 40.9, 28.9; IR ν_{max} (neat) 3303, 2964, 2939, 2874, 2828, 1605, 1515, 1464, 1438, 1345, 1245, 1219, 1144, 1060, 1010, 990, 862, 791, 766, 610 cm^{-1}; MS (ESI, +ve) *m/z* 322 [$(M+Na)^+$, 100%], 282 (46); HRMS (ESI, +ve) $(M+Na)^+$ calcd for $C_{18}H_{21}NNaO_3$ 322.1419, found 322.1420.

(4,5-Dimethoxy-2-(1-methyl-1*H*-indol-7-yl)phenyl)methanol (28). A magnetically stirred solution of compound **2** (20 mg, 0.07 mmol) in CH_2Cl_2 (1 mL) was treated with manganese(IV) oxide (32 mg, 0.368 mmol) at 22 °C. The ensuing mixture was stirred at 22 °C for 72 h then filtered through a pad of diatomaceous earth. The filtrate thus obtained was concentrated under reduced pressure to give a brown oil and subjection of this to flash chromatography (silica, diethyl ether elution) followed by concentration of

the appropriate fractions (R_f = 0.3) gave compound **28** (15 mg, 76%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (d, *J* = 7.5 Hz, 1H), 7.13-7.09 (complex m, 2H), 6.98-6.95 (complex m, 2H), 6.86 (s, 1H), 6.53 (d, *J* = 3.1 Hz, 1H), 4.38 (s, 2H), 3.98 (s, 3H), 3.85 (s, 3H), 3.24 (s, 3H) (signal due to hydroxyl group proton obscured/not observed); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.6, 147.4, 134.1, 132.1, 130.8, 129.6, 123.8(1), 123.7(6), 120.5, 119.1, 113.9, 110.6, 101.1, 63.1, 56.0, 55.9, 35.7 (one signal obscured or overlapping); IR ν_{max} (KBr) 3493, 2999, 2936, 2847, 1606, 1523, 1509, 1464, 1443, 1342, 1318, 1243, 1212, 1142, 1118, 1074, 998, 729 cm^{-1}; MS (EI, 70 eV) *m/z* 297 ($M^{+\bullet}$, 100%); HRMS (EI, 70 ev) $M^{+\bullet}$ calcd for $C_{18}H_{19}NO_3$ 297.1365, found 297.1369.

4,5-Dimethoxy-2-(1-methyl-1*H*-indol-7-yl)benzaldehyde (29). A mixture of molecular sieves (20 mg of powdered, 4 Å material), diatomaceous earth (20 mg), activated magnesium silicate (20 mg of 60-100 mesh material), sodium acetate (14 mg, 0.171 mmol) and pyridinium chlorochromate (25 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) was stirred at 22 °C for 0.5 h then treated with a solution of compound **2** (20 mg, 0.067 mmol) in CH_2Cl_2 (1 mL). The ensuing mixture was stirred at 22 °C for 17 h then diluted with diethyl ether (20 mL) and the resulting mixture filtered through a pad of diatomaceous earth. The filtrate thus obtained was concentrated under reduced pressure to give a clear, colorless oil that was subjected to flash chromatography (silica, 3:7 v/v diethyl ether/pentane elution) and thereby affording two fractions, A and B.

Concentration of fraction A (R_f = 0.4 in 3:7 v/v diethyl ether/pentane) afforded the title compound **29** (7 mg, 35%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.62 (s, 1H), 7.69 (dd, *J* = 7.5 and 1.0 Hz, 1H), 7.55 (s, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.03

(dd, J = 7.5 and 1.0 Hz, 1H), 6.98 (d, J = 3.1 Hz, 1H), 6.93 (s, 1H), 6.57 (d, J = 3.1 Hz, 1H), 4.02 (s, 3H), 3.94 (s, 3H), 3.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.1, 153.0, 149.1, 139.1, 134.8, 131.1, 129.6, 128.6, 125.0, 121.2, 120.9, 118.9, 113.8, 107.7, 101.4, 56.3, 56.1, 36.1; IR ν_{max} (KBr) 2937, 2848, 1676, 1596, 1508, 1463, 1395, 1349, 1284, 1256, 1243, 1217, 1141, 1118, 1072, 911, 730 cm^{-1}; MS (EI, 70 eV) *m/z* 295 ($M^{+\bullet}$, 100%), 280 (15), 266 (22), 252 (15), 236 (17), 86 (12), 84 (19); HRMS (EI, 70 ev) $M^{+\bullet}$ calcd for $C_{18}H_{17}NO_3$ 295.1208, found 295.1208.

Concentration of fraction B (R_f = 0.1 in 3:7 v/v diethyl ether/pentane) afforded compound **28** (1 mg, 5%) as a clear, colorless oil. This material was identical, in all respects, with that obtained as described above.

X-ray Crystallographic Studies

Crystallographic Data

Compound **2**. $C_{18}H_{21}NO_3$, M = 299.37, T = 150 K, triclinic, space group $P\bar{1}$, Z = 2, a = 8.0070(4) Å, b = 8.6147(3) Å, c = 12.8193(6) Å; α = 84.737(4)°, β = 79.243(4)°, γ = 63.307 (4)°; V = 776.10(7) Å^3, D_x = 1.281 g cm^{-3}, 3055 unique reflections ($2\theta_{max}$ = 144.6°), R = 0.034 [for 2898 reflections with $I > 2.0\sigma(I)$]; Rw = 0.091 (all data), S = 1.00.

Compound **18**. $C_{16}H_{13}NO_7$, M = 331.27, T = 150 K, monoclinic, space group $P2_1/c$, Z = 4, a = 8.92045(5) Å, b = 11.34780(6) Å, c = 14.26284(8) Å; β = 102.5075(6)°; V = 1409.53(1) Å^3, D_x = 1.561 g cm^{-3}, 2837 unique reflections ($2\theta_{max}$ = 145.4°), R = 0.038 [for 2815 reflections with $I > 2.0\sigma(I)$]; Rw = 0.100 (all data), S = 1.08.

Compound **21**. $C_{16}H_{13}NO_6$, M = 315.27, T = 150 K, triclinic, space group $P\bar{1}$, Z = 2, a = 6.6160(3) Å, b = 9.3462(3) Å, c = 11.3791(5) Å; α = 86.286(3)°, β = 89.459(4)°, γ =

85.502(3)°; V = 699.97(5) Å^3, D_x = 1.496 g cm^{-3}, 3536 unique reflections ($2\theta_{max}$ = 59.8°), R = 0.043 [for 2887 reflections with $I > 2.0\sigma(I)$]; Rw = 0.123 (all data), S = 1.02.

Compound **27**. $C_{17}H_{17}NO_3$, M = 283.33, T = 150 K, monoclinic, space group $P2_1/n$, Z = 8, a = 17.4725(2) Å, b = 8.6511(1) Å, c = 18.9278(2) Å; β = 96.8003(8)°; V = 2840.93(6) Å^3, D_x = 1.325 g cm^{-3}, 5617 unique reflections ($2\theta_{max}$ = 144.8°), R = 0.038 [for 5080 reflections with $I > 2.0\sigma(I)$]; Rw = 0.107 (all data), S = 1.00.

Structure Determination

The images for compound **21** were measured on a diffractometer (Mo Kα, graphite monochromator, λ = 0.71073 Å) fitted with an area detector and the data extracted using CrysAlis PRO.[19] Images for compounds **2**, **18** and **27** were measured on a diffractometer (Cu Kα, mirror monochromator, λ = 1.54184 Å) fitted with an area detector and the data extracted using the CrysAlis PRO.[19] The structure solutions for all four compounds were solved by direct methods (SIR92)[20] then refined using the CRYSTALS program package.[21]Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC nos. 1543361, 1543362, 1543363 and 1543364). 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.

AChE Inhibition Assay.

AChE activity was assayed according to the method of Ellman and co-workers.[22] Compounds **1**, **2**, **17-19, 21-24**, **27** and galanthamine were prepared in DMSO to 50 mM, then serially diluted one-in-three in DMSO to give concentrations ranging from 50 mM

to 30 nM. 2 µl of compound at each concentration was added to 98 µL of 5-5'-dithiobis-(2-nitrobenzoic acid) (510 µM in phosphate buffer (100 mM Na_2HPO_4 pH 7.4)) and 60 µL of *Electrophorus electricus* AChE (Type V-S, 1 nM in phosphate buffer supplemented with 1 mg/ml bovine serum albumin). The enzymatic reaction was initiated by addition of 40 µl of acetylthiocholine iodide (1.15 mM in phosphate buffer) and progress was monitored by measuring absorbance at 412 nm for six minutes. Measurements were conducted in triplicate. Velocities were determined by linear regression, and were corrected for non-enzymatic substrate hydrolysis. The concentration of compound required to reduce AChE activity to 50% of a neat DMSO control (IC_{50}) was calculated by fitting a sigmoidal dose-response curve to percentage activity using GraphPad Prism.

ASSOCIATED CONTENT

Supporting Information

Data derived from the single-crystal X-ray analyses of compounds **2**, **18**, **21** and **27**; ^{1}H and ^{13}C NMR spectra of compounds **1**, **2**, **5-7**, **11**, **14-25** and **27-29**. AChE inhibition curves for compounds **1**, **2**, **17-19, 21-24** and **27**. This material is available free-of-charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Jinan University, the Australian Research Council and the Institute of Advanced Studies for financial support. HSK and GJC are the grateful recipients of Scholarships provided by the Australian Government.

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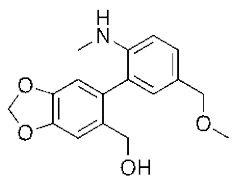
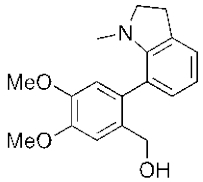
TOC Graphic



zephycandidine III
(**1**)



lycosinine A
(**2**)