Rapid synthesis and zebrafish evaluation of a phenanthridine-based small molecule library

Post-print of peer-reviewed article published by the Royal Society of Chemistry.
Published article available at: http://dx.doi.org/10.1039/C0OB00449A

Cite as:
Donaldson, L. R., Wallace, S., Haigh, D., Patton, E., & Hulme, A. N. (2011). Rapid synthesis and zebrafish evaluation of a phenanthridine-based small molecule library. Organic & Biomolecular chemistry, 9, 2233–2239.

Manuscript received: 19/07/2010; Accepted: 20/12/2010; Article published: 21/12/2010

Rapid synthesis and zebrafish evaluation of a phenanthridine-based small molecule library**

Lauren R. Donaldson,[1] Stephen Wallace,[1] David Haigh,[2,4] E. Elizabeth Patton[3,4] and Alison N. Hulme[1,*]

[1]EaStCHEM, School of Chemistry, Joseph Black Building, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, UK.

[2]GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, UK.

[3]Edinburgh Cancer Research Centre & MRC Human Genetics Unit, The University of Edinburgh, Crewe Road South, Edinburgh, UK.

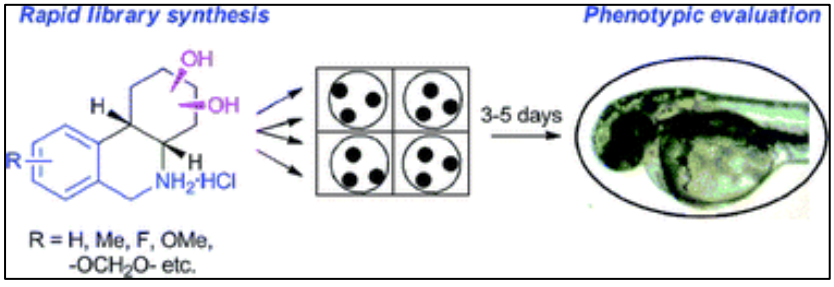
[4]Current address: Centre for Cancer Research & Cell Biology, Queens University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK.

[*]Corresponding author; Alison.Hulme@ed.ac.uk, tel.: + 44 131 650 4711, fax: + 44 131 650 4743

[**]We thank the EPSRC (DTA award to LRD), the Society for Chemical Industries (Messel Scholarship to LRD), Medical Research Scotland, the Medical Research Scotland MRC and GlaxoSmithKline for financial support of this work. LRD also thanks Corina Anastasaki and Jennifer Richardson for their assistance during the zebrafish screening process.

Supporting information:
Electronic Supplementary Information (ESI) available: [Experimental details for the preparation and characterisation of all the other library members are given in the supporting information]. See http://dx.doi.org/10.1039/C0OB00449A

Graphical abstract:



Abstract

A Heck cyclisation approach is described for the rapid synthesis of a library of natural product-like small molecules, based on the phenanthridine core. The synthesis of a range of substituted benzylamine building blocks and their incorporation into the library is reported, together with a highly selective *cis*-dihydroxylation protocol that enables access to the target compounds in an efficient manner. Biological evaluation of the library using zebrafish phenotyping, has led to the discovery of compound **20c**, a novel inhibitor of early-stage zebrafish embryo development.

Introduction

Forward chemical genetics techniques seek to emulate the success of classical genetics by using small molecules to probe the function of gene-products and thus reveal information about biological processes.[1] Typically, a small molecule library is screened against a cellular or embryonic system and the induction of any abnormal phenotype is identifed. The protein target that has been modulated may then be deduced;[2] thus providing both information on its function and a small molecule modulator from one process. The ongoing search for modulators of all "druggable" targets puts heavy demands on the sourcing of small molecule libraries which may be obtained from in-house compound collections,[3] commercially acquired combinatorial libraries,[1] or even virtual libraries.[4] However, many such compound collections suffer from a somewhat reduced scope, which has led to renewed interest in libraries based on the diverse core-structures of natural products.[5] These complex architectures have evolved to fulfil specific biological functions and, as a result of the close evolutionary relationship shared by the genomes of all organisms, bind to proteins similar to human proteins.[5] Natural products are therefore unmatched in terms of their inherent bioactivity and their ability to probe chemical space; thus they represent excellent, biologically-validated scaffolds upon which to base chemical library design.

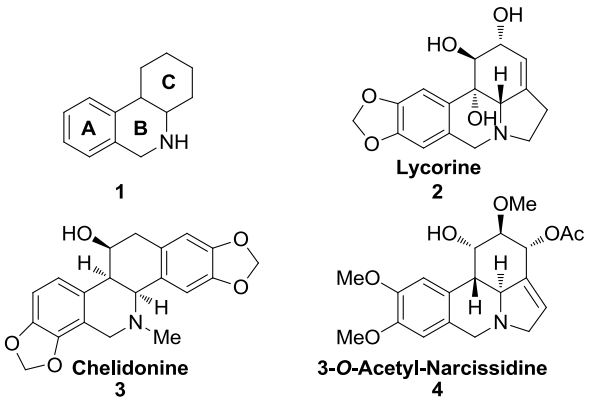
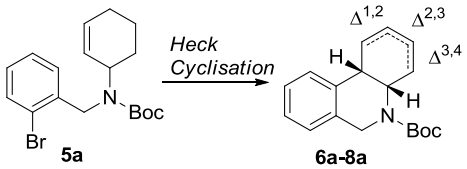
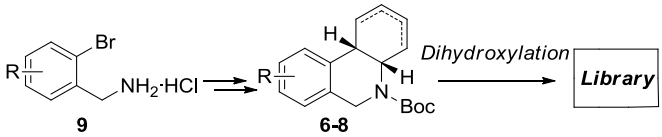


Figure 1. The phenanthridine core **1** and biologically active phenanthridine natural products **2**-**4**.



Scheme 1. Heck cyclisation approach to the phenanthridine core.[9] **6a**=$\Delta^{1,2}$; **7a**=$\Delta^{2,3}$; **8a**=$\Delta^{3,4}$. Reagents and Conditions: Cationic: Herrmann-Beller palladacycle (5 mol%), Ag_2CO_3, DMF, 140°C, (99%, **6a**:**7a**:**8a** 83:15:2); Neutral: Herrmann-Beller palladacycle (5 mol%), $MeNCy_2$, DMF, 140°C (95%, **6a**:**7a**:**8a** 36:38:26); or $Pd_2(dba)_3$, $^tBu_3P{\bullet}HBF_4$ (10 mol%), $MeNCy_2$, MeCN, r.t.-50°C, (95%, **6a**:**7a**:**8a** 34:37:29).

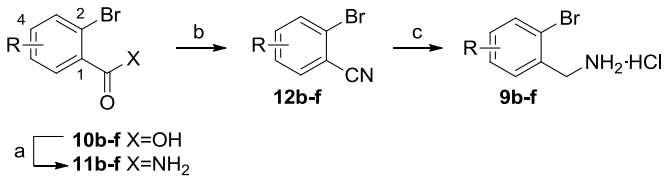
The phenanthridine core **1** (Figure 1) lies at the heart of many bioactive natural products including the antiviral lycorine **2**,[6] the tubulin polymerisation inhibitor chelidonine **3**,[7] and the insect anti-feedant 3-*O*-acetyl-narcissidine **4**.[8] We recently disclosed methodology for the efficient Heck cyclisation of carbamate precursor **5a** (Scheme 1), to afford *cis*-ring fused phenanthridine double bond isomers **6a** ($\Delta^{1,2}$ alkene), **7a** ($\Delta^{2,3}$ alkene) and **8a** ($\Delta^{3,4}$ alkene) in excellent yield.[9] By combining this methodology with a range of benzylamine building blocks **9** (Scheme 2), rapid access to a collection of A-ring modified phenanthridine analogues should be achieved, each with an alkene handle suitable for further functionalisation. Due to the prevalence of hydroxylated C-ring components in bioactive phenanthridine natural products,[6-8,10] subsequent functionalisation of the alkene isomers **6-8** was envisaged using a dihydroxylation protocol. In order to evaluate the resultant novel natural-product inspired small molecules, library synthesis was coupled with phenotypic evaluation in embryonic zebrafish.[11]



Scheme 2. Proposed library synthesis.

Library Synthesis

Initial efforts focused on the synthesis of Heck cyclisation precursors incorporating a range of benzylamine building blocks. To this end, standard transformations were employed for the synthesis of benzylamines **9b-f** from their commercially available benzoic acid counterparts **10b-f** (Table 1).

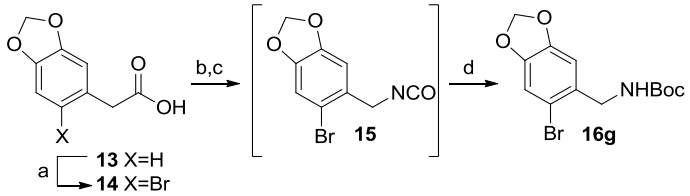


Reagents and Conditions: a) i) $SOCl_2$, 60°C, 3 h; ii) NH_4OH, r.t., 16 h; b) $SOCl_2$, 60°C, 3 h; c) i) $LiAlH_4$, $AlCl_3$, Et_2O, Δ, 16 h; ii) HCl in Et_2O.

Entry	10	R=	Yield 11 (%)	Yield 12 (%)	Yield 9 (%)
1	**b**	4-Me	95	73	80
2	**c**	4-F	94	97	70
3	**d**	5-OMe	73	99	65
4	**e**	4,5-di-OMe	99	88	74
5	**f**	3,4-CH=CH-CH=CH-	99	95	55

Table 1. Synthesis of amine building blocks **9b-f**.

To access the corresponding piperonyl building block (R= 4,5 -OCH_2O-), a high yielding, two-pot Curtius rearrangement approach was used. This allowed direct access to the *N*-Boc protected derivative of this analogue **16g** *via* isocyanate intermediate **15** (Scheme 3).[12]

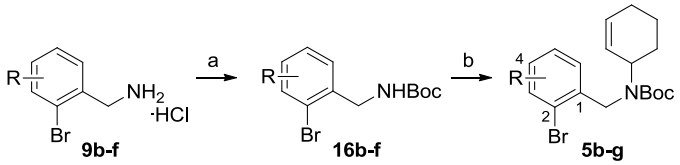


Scheme 3. Synthesis of piperonyl cyclisation precursor **16g**.

Reagents and Conditions: a) DBDMH, 4 M NaOH, H_2O, r.t., 16 h, 91%; b) dppa, Et_3N, CH_2Cl_2, 0°C → r.t., 0.5 h; c) i) silica plug filtration (partial conversion); ii) PhMe, 110°C, 1 h (complete conversion); d) ^{t}BuOH, 80°C, 16 h, 50% over 3 steps.

N-Boc-protection of benzylamine hydrochlorides **9b-f**, followed by alkylation of the

corresponding carbamates **16b-g** gave the desired cyclisation precursors **5b-g** in good to excellent yield (Table 2).



Reagents and Conditions: a) Boc_2O, Et_3N, CH_2Cl_2, 16 h; b) NaH, 3-bromocyclohexene, DMF, 0°C-r.t., 16 h.

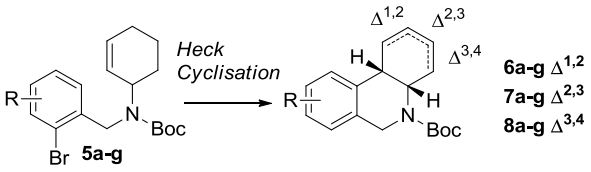
Entry	Substrate	R=	Yield 16 (%)	Yield 5 (%)
1	**b**	4-Me	99	70
2	**c**	4-F	88	71
3	**d**	5-OMe	85	72
4	**e**	4,5-di-OMe	99	84
5	**f**	3,4-CH=CH-CH=CH-	90	86
6	**g**	4,5-O(CH_2)O-	-	75

Table 2. Synthesis of Heck cyclisation precursors **5b-g**.

With the cyclisation precursors **5b-g** in hand we studied their reaction under the neutral Herrmann-Beller palladacycle catalysed Heck cyclisation conditions we had previously developed (Table 3).[9] We found that for the majority of substrates, these conditions promoted cyclisation to the expected mixture of double bond regioisomers **6-8**. However, conversions were lower in all cases than that exhibited by the parent Boc substrate **5a** (95% in 3 h),[9] and reaction times were longer, especially with the electron-rich aromatics (Table 3, entries 2, 4, 5, 8). For piperonyl substrate **5g** (Table 3, entry 8) a significant amount of the debrominated starting material was recovered, suggesting this substrate was particularly sluggish to react under the palladacycle-catalysed conditions. Fortunately, application of our previously developed low temperature ($(^tBu_3P)_2Pd$ conditions (Table 3, entry 9),[9] enabled the recovery of the desired product mixture **6-8g** in 80% yield at r.t., and 99% yield at 50°C (Table 3, entry 10). The regioisomeric mixtures of phenanthridine double bond isomers were used in the subsequent investigation of the dihydroxylation of the C-ring.[13]

Molecular models suggested a cupped conformation for the *cis*-ring junction phenanthridines **6-8**;

thus it was anticipated that dihydroxylation would be more favoured on the convex face. In order to assess the likely diastereoselectivity of the dihydroxylation reaction across the library, parent phenanthridines **6-8a** were separated by HPLC[14] and subjected individually to standard dihydroxylation conditions (Scheme 4).[15] Quantitative conversions to the corresponding diols were achieved for all three isomers, with diastereoselectivities from 83:17-85:15 as determined by ^{1}H NMR. The relative stereochemistry of the major product was confirmed as the exo-diol using 2D NMR studies.[16]

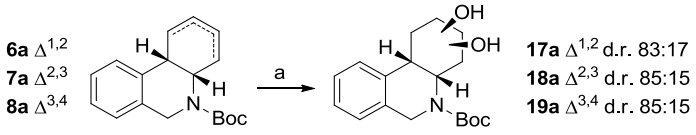


Reagents and Conditions: aryl halide (1 eq), $MeNCy_2$ (4 eq), Herrmann-Beller palladacycle (5 mol%), DMF, 140°C.

Entry	5	R=	t (h)	Yield 6-8 (%)	Ratio[a] 6:7:8
1	**a**	H	3	95	36:38:26
2	**b**	4-Me	12	76	26:57:17
3	**c**	4-F	5	72	27:44:29
4	**d**	5-OMe	6	73	36:44:20
5	**e**	4,5-di-OMe	12	75	41:32:27
6	**f**	3,4-CH=CH-CH=CH-	5	74	42:42:16
8	**g**	4,5-O(CH_2)O-	24	59 (32)[b]	41:26:33
9	**g**	4,5-O(CH_2)O-	18	80 (11)[c,d]	18:46:36
10	**g**	4,5-O(CH_2)O-	18	99[e]	37:39:24

[a] Ratio identified from ^{1}H NMR of isolated product mixture. [b] 32% debrominated material recovered. [c] $Pd_2(dba)_3$ (5 mol%), $^tBu_3P$•HBF_4 (10 mol%), $MeNCy_2$ (4 eq), MeCN, r.t. [d] Starting material recovered. [e] As entry 9 but at 50°C.

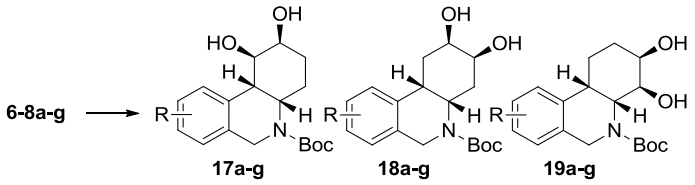
Table 3. Heck cyclisation of carbamates **5a-g**.



Scheme 4. *Cis*-dihydroxylation of phananthridines **6a-8a**.

Reagents and Conditions: a) OsO_4, NMO, THF/H_2O, r.t., 18 h (**17a** 99%; **18a** 99%; **19a** 99%).

When this dihydroxylation protocol was applied to phenanthridine double bond isomer mixtures **6-8a-g** the corresponding diol mixtures **17-19a-g** were afforded in good to excellent yield (Table 4). In keeping with the high diasteromeric ratio we obtained for the *cis*-dihydroxylation of individual carbamates **6-8a**, we observed the *exo*-diols as the major products in all cases.[18]



Reagents and Conditions: OsO_4, NMO, THF/H_2O, r.t., 18 h.

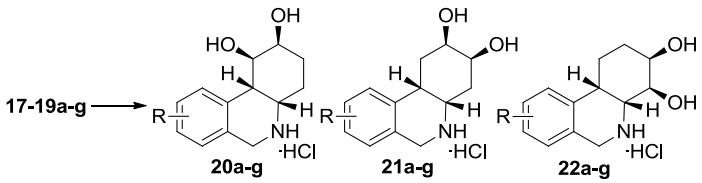
Entry	6-8	R=	Yield 17-19 (%)[a]
1	**a**	H	70
2	**b**	4-Me	78
3	**c**	4-F	82
4	**d**	5-OMe	95
5	**e**	4,5-di-OMe	70
6	**f**	3,4-CH=CH-CH=CH-	87
7	**g**	4,5-O(CH_2)O-	87[b]

[a] Isolated yield of mixture following flash chromatography. [b] includes 2% $\Delta^{2,3}$ *endo-syn* diol product as characterised by 2D NMR studies.[15]

Table 4. *Cis*-dihydroxylation of phenanthridines **6-8a-g**.

Following dihydroxylation, samples of the three major products *exo-syn* diols **17**, **18** and **19** were successfully isolated by silica-gel chromatography or HPLC separation. However, we were unable to separate the 4-methyl diol products **17b-19b** under a range of conditions. Similarly, the HPLC fractions for the $\Delta^{1,2}$ and $\Delta^{2,3}$ dimethoxy analogues **17e** and **18e** overlapped significantly, and thus the $\Delta^{2,3}$ sample had significant $\Delta^{1,2}$ contamination. For these two substrates, mixtures of the appropriate compounds were carried through to the biological testing stage.

For the last step in our library synthesis, we took the diol mixtures and isolated compounds and converted each into the hydrochloride salts, with yields as summarised in **Table 5**. This afforded 18 isolated compounds and 5 mixtures for biological testing.



Reagents and Conditions: i) TFA, CH_2Cl_2, 1 h, r.t.; ii) NaOH, adjust to pH 9; iii) HCl in Et_2O, 0°C.

Entry	**17-19**	**R=**	**Yield 20-22 (%)**[a]	**20 (%)**	**21 (%)**	**22 (%)**
1[b]	**a**	H	-	86	60	53
2	**b**	4-Me	69	-	-	-
3	**c**	4-F	99	99	86	74
4	**d**	5-OMe	89	82	92	84
5	**e**	4,5-di-OMe	87	88	60[c]	60
6[b]	**f**	3,4-CH=CH-CH=CH-	-	67	61	69
7	**g**	4,5-O(CH_2)O-	69	61	81	73

[a] Performed on mixture. [b] No diol mixture was deprotected for these analogues as significant isolated material was available as compared to the other substrates. [c] Approximately 1:1 mixture $\Delta^{1,2}$:$\Delta^{2,3}$.

Table 5. Boc-deprotection to phenanthridinium hydrochloride library.

Zebrafish Phenotyping

Zebrafish (*Danio rerio*) embryos are transparent for the first five days post fertilisation (dpf), and develop outwith the mother's body, permitting visual inspection of their anatomical development to take place.[11] Fertilised embryos were collected and arrayed in 96 well plates (3 eggs per well; from 6 hours post fertlization, hpf) containing embryo buffer (200 μL), and then library members in their buffer/0.5% DMSO stock solutions (10-100 μM for the mixtures, and 1-50 μM for the isolated compounds), were exchanged for the buffer, one compound or mixture per well.[19] The embryos were incubated at 28.5°C and examined visually for developmental defects[11] using a light-dissecting microscope at 1, 2, 3 and 4 dpf.

Our preliminary screening was performed using the mixtures of diols where available, so that precious isolated material was not wasted. Fluorinated compound mixture **20c-22c** was identified as causing reproducible embryo death by 1 dpf, and so we examined each component of the mixture individually. Analogue **20c** ($\Delta^{1,2}$ isomer) was found to interfere reproducibly with zebrafish development (as assessed by the head-trunk angle, which may be used as a measure of

developmental hours[20]) at concentrations of 100, 50 and 20 μM. By almost 70 hpf all fish were delayed developmentally in a dose-dependant manner, and exhibited lower than normal pigmentation (Figure 2). At 5 dpf, the yolk sack of the fish treated with 100 μM **20c** was significantly larger than that of the control, providing further evidence for delayed development (Figure 3). No notable difference from the control was observed in the fish exposed to lower drug concentrations at 5 dpf. None of the other compound mixtures or isolated compounds exhibited any specific phenotype when tested on the zebrafish embryos.

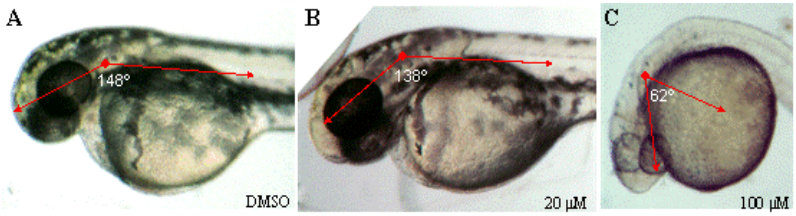


Figure 2. Zebrafish embryos treated with compound **20c** are delayed during embryonic development. Zebrafish embryos were treated with **20c** for three days, and screened for toxicity or developmental phenotypes. One compound, **20c**, caused a dose-dependent developmental delay. Control treated embryos appeared normal (**A**), and developed pigmentation (dark coloring). The head-trunk angle, used as a measure of developmental hours,[20] indicates that the control animals are aged at approximately 61 hpf (148º angle from middle of the ear and eye to the notochord). By comparison, zebrafish treated with 20 μM concentrations of **20c** (**B**) appear correctly formed but slightly delayed in development by about 9 h (head-trunk angle 138º). Embryos treated with 100 μM concentrations of **20c** (**C**) are strongly delayed for development, as indicated by a low head-trunk angle (62º; equivalent to approximately 24 hpf).

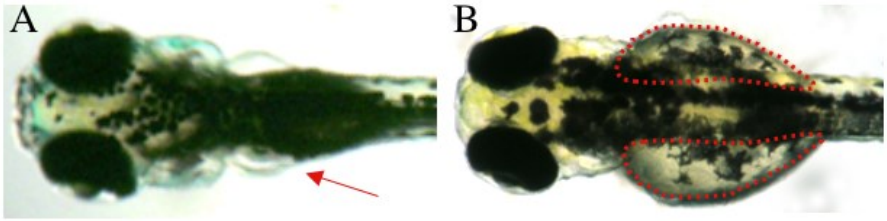


Figure 3. Zebrafish embryos treated with 100 μM compound **20c** at 5 dpf. Some of the embryos treated with **20c** underwent development at a similar rate to the control fish, but did not consume their yolk (**B**, red dotted line), as compared to the control treated fish (**A**, red arrow). This can indicate developmental defects in intestinal development, or a more general toxicity to embryonic development.

Conclusions

We have demonstrated that libraries based upon the core structure of natural products can be fruitful in the chemical genetics quest for the discovery of novel small molecule probes for biological processes. The diversity of such libraries can be enhanced by exploiting multi-product-generating reactions such as the Heck cyclisation, in conjunction with other methodologies that enable access to individual library members. Through the development of various aryl cyclisation precursors and a highly selective dihydroxylation protocol, we were able to rapidly synthesise a small library of novel phenanthridine analogues suitable for biological testing. The preliminary evaluation of this library using zebrafish phenotyping has led to the discovery of a novel inhibitor of zebrafish embryonic development. Further biological investigations including cell-based assays and affinity chromatography followed by protein micro-sequencing,[21] should enable identification of the target protein and provide an understanding of the mode of action of compound **20c** at a molecular level.

Experimental

General methods: All non-aqueous reactions were carried out under an atmosphere of nitrogen using flame- or oven-dried glassware that was cooled in a dessicator prior to use. Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Dichloromethane (CH_2Cl_2) and triethylamine (Et_3N) were distilled from and stored over calcium hydride under a nitrogen atmosphere. Tetrahydrofuran (THF) was distilled from sodium/benzophenone and stored under a nitrogen atmosphere. Diethyl ether (Et_2O) was dried and purified by passage through activated alumina columns using a solvent purification system from www.glasscontour.com. Anhydrous DMF and acetonitrile (MeCN) were used as supplied by BakerDRY. Saturated aqueous solutions of inorganic salts are represented as (volume, sat. aq.). ^1H and ^{13}C NMR spectra were obtained on Bruker instuments at the stated frequency; *J* values are in Hz. Infra-red spectra were recorded using a Biorad FTS-7 or Perkin-Elmer Paragon 1000 FT-IR spectrometer as thin films. Electron impact (EI) mass spectra were obtained using a Finnigan 4500 mass spectrometer, and electrospray (ESI) and fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS50TC mass spectrometer. Melting points were determined on a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected. Flash chromatography was carried out using Merck Kieselgel 60 (Merck 9385) under positive pressure by means of an airline or hand pump. Eluent compositions are quoted as $^v/_v$ ratios. High performance liquid chromatography (HPLC) was carried out using a Gilson instrument fitted with a refractive index detector, using a Spherisorb silica column (length 250 mm, i.d. 20.0 mm, particle size 5 μm). Flow rates are a quoted for each separation in mL min^{-1}.

2-Bromo-4-fluorobenzamide 11c: A mixture of 2-bromo-4-fluoro benzoic acid (1.00 g, 4.57 mmol) and thionyl chloride (15 mL) was heated at 60°C for 3 h. The thionyl chloride was removed under reduced pressure and the residue was dissolved in NH_4OH (15 mL, conc.) and stirred for 16 h at r.t. The reaction mixture was filtered and the precipitate dried on the high vacuum line for several hours to afford afford amide **11c** as a colourless solid (940 mg, 94%).

R_f [CH_2Cl_2:MeOH, 95:5] = 0.4; MP 155°C (H_2O); υ_{max} ($CHCl_3$)/cm^{-1} 3400 (NH), 1639 (C=O); ^1H NMR δ (360 MHz, CD_3OD) 7.50 (1H, dd, *J* 8.4, 5.8, Ar*H*), 7.46 (1H, dd, *J* 8.6, 2.6, Ar*H*), 7.22 (1H, td, *J* 8.4, 2.6, Ar*H*); ^{13}C NMR δ (90.6 MHz, CD_3OD) 172.6 (C), 164.4 (d, *J* 252.1, C), 136.5 (C), 131.7 (d, *J* 9.0, CH), 121.7 (d, *J* 25.1, CH), 121.2 (d, *J* 9.9, C), 115.9 (d, *J* 21.7, CH); *m/z* (FAB, 3-NOBA) 220 ([^{81Br}M+H]$^+$, 64 %), 218 ([^{79Br}M+H]$^+$, 66), 167 (12), 165 (11), 136 (100); HRMS (EI) Found: [^{81Br}M]$^+$, 218.9511. $C_7H_5ON^{81}BrF$ requires 218.9513. Found: [^{79Br}M]$^+$, 216.9528. $C_7H_5ON^{79}BrF$ requires 216.9533.

2-Bromo-4-fluorobenzonitrile 12c: A mixture of amide **11c** (940 mg, 4.30 mmol) and thionyl chloride (6 mL) was refluxed at 60°C for 3 h. The reaction was concentrated under reduced

pressure to afford nitrile **12c** as a colourless solid (840 mg, 97%).

R_f [hexane:EtOAc, 3:1] = 0.78; MP 76°C (EtOH); υ_{max} ($CHCl_3$)/cm^{-1} 2228 (CN); ^{1}H NMR δ (360 MHz, CD_3OD) 7.88 (1H, dd, *J* 8.6, 5.4, Ar*H*), 7.69 (1H, dd, *J* 8.3, 2.5, Ar*H*), 7.35 (1H, td, *J* 8.6, 2.5, Ar*H*); ^{13}C NMR δ (90.6 MHz, CD_3OD) 166.4 (d, *J* 259.3, C), 138.0 (d, *J* 10.1, CH), 127.6 (d, *J* 10.3, C), 122.5 (d, *J* 26.1, CH), 117.6 (C), 117.3 (d, *J* 22.9, CH), 113.5 (C); *m/z* (FAB, 3-NOBA) 202 ([^{81Br}M+H]$^+$, 3 %), 200 ([^{79Br}M+H]$^+$, 2), 154 (64), 121 (11); HRMS (EI) Found: [^{81Br}M]$^+$, 200.9407. $C_7H_3{}^{81}BrFN$ requires 200.9407. Found: [^{79Br}M]$^+$, 198.9422. $C_7H_3{}^{79}BrFN$ requires 198.9427.

2-Bromo-4-fluorobenzylamine hydrochloride 9c: To a suspension of $LiAlH_4$ (95 mg, 2.50 mmol) in Et_2O (5 mL) was added $AlCl_3$ (334 mg, 2.50 mmol) and the mixture was cooled to 0°C and stirred for 10 mins. The reaction was warmed to r.t., nitrile **12c** (249 mg, 1.25 mmol) was added portionwise, the reaction was stirred for 30 mins and then heated at 40°C for 18 h. The reaction was quenched by the addition of $Na_2SO_4.5H_2O$ portionwise, filtered and the filtrate stirred vigorously with potassium sodium tartrate (100 mL, sat. aq.) for 1 h. The Et_2O layer was separated and the aqueous phase extracted with Et_2O (3 × 60 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (1 mL), and HCl in Et_2O (20.0 mL, 1 M in Et_2O) was added. The precipitate was removed by filtration and dried to afford amine hydrochloride **9c** as a colourless solid (210 mg, 70%).

MP 251°C (Et_2O); ^{1}H NMR δ (250 MHz, CD_3OD) 7.63 (1H, dd, *J* 8.8, 6.0, Ar*H*), 7.58 (1H, dd, *J* 8.3, 2.8, Ar*H*), 7.28 (1H, td, *J* 8.5, 2.8, Ar*H*), 4.29 (2H, s, CH_2); ^{13}C NMR δ (62.9 MHz, CD_3OD) 163.5 (d, *J* 252.4, C), 133.1 (d, *J* 9.0, CH), 129.6 (C), 125.3 (d, *J* 10.3, C), 120.9 (d, *J* 25.1, CH), 115.9 (d, 21.6, CH), 42.8 (CH_2); *m/z* (FAB, 3-NOBA) 206 ([^{81Br}M+H]$^+$, 14 %), 204 ([^{79Br}M+H]$^+$, 17), 189 (37), 187 (40), 149 (47); HRMS (ESI+, CH_2Cl_2/MeOH/NH_4OAc) Found: [M+H]$^+$, 203.9820. $C_7H_8NF^{79}Br$ requires 203.9819. Free Amine: R_f [CH_2Cl_2:MeOH, 95:5] = 0.46; υ_{max} ($CHCl_3$)/cm^{-1} 3285 (NH), 2926, 1597, 1484; ^{1}H NMR δ (250 MHz, $CDCl_3$) 7.33 (1H, dd, *J* 8.5, 6.0, Ar*H*), 7.26 (1H, dd, *J* 8.3, 2.5, Ar*H*), 6.98 (1H, td, *J* 8.3, 2.5, Ar*H*), 3.85 (2H, s, CH_2); ^{13}C NMR δ (62.9 MHz, $CDCl_3$) 160.6 (d, *J* 249.3, C), 137.4 (C), 129.1 (d, *J* 7.9, CH), 122.6 (d, *J* 9.6, C), 119.1 (d, *J* 24.8, CH), 113.9 (d, *J* 20.8, CH), 45.4 (CH_2).

2-Bromo-4-fluorobenzyl *tert*butylcarboxamide 16c: A solution of amine hydrochloride **9c** (167 mg, 0.69 mmol) and Et_3N (195 µl, 1.39 mmol) in CH_2Cl_2 (8 mL) was stirred for 10 mins then cooled to 0°C. Boc_2O (167 mg, 0.76 mmol) was added and the reaction mixture was allowed to warm to r.t. and stirred for 4 h. The reaction was diluted with CH_2Cl_2 (15 mL) and washed with NaCl (3 × 15 mL, sat. aq.). The organics were combined, dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography (hexane:EtOAc, 10:1) to afford carbamate

16c as a colourless oil (184 mg, 88%).

R_f [hexane:EtOAc, 3:1] = 0.85; υ_{max} ($CHCl_3$)/cm^{-1} 3343 (NH), 1696 (C=O); ^{1}H NMR δ (250 MHz, $CDCl_3$) 7.35 (1H, dd, *J* 8.3, 6.0, Ar*H*), 7.27 (1H, dd, *J* 8.3, 2.5, Ar*H*), 6.99 (1H, dt, *J* 8.3, 2.5, Ar*H*), 5.11 (1H, br s, *CH*N), 4.33 (2H, d, *J* 6.3, CH_2Ar), 1.44 (9H, s, 3×CH_3); ^{13}C NMR δ (62.9 MHz, $CDCl_3$) 163.4 (C), 157.5 (d, *J* 245.8, C), 133.8 (C), 130.5 (d, *J* 7.8, CH), 123.2 (d, *J* 9.6, C), 119.8 (d, *J* 24.5, CH), 114.4 (d, *J* 20.9, C), 79.6 (C), 44.0 (CH_2), 28.2 (3×CH_3); *m/z* (EI) 306 ([^{81Br}M+H]$^+$, 1 %), 304 ([^{79Br}M+H]$^+$, 1), 248 (14), 246 (13), 189 (17), 187 (18) 168 (100); HRMS (EI) Found: [^{79Br}M]$^+$, 303.0279. $C_{12}H_{15}{}^{79}BrFNO_2$ requires 303.0265.

2-Bromo-4-fluorobenzyl cyclohex-2-enyl-carbamic acid *tert*-butyl ester 5c: A solution of Boc carbamate **16c** (175 mg, 0.56 mmol) in DMF (4 mL) was cooled to 0°C and NaH (46 mg, 60% dispersion in mineral oil, 1.15 mmol) was added. The reaction was warmed to r.t. for 40 mins, then cooled to 0°C and and 3-bromocyclohexene (133 µl, 1.15 mmol) was added dropwise. The reaction allowed to warm to r.t. and stirred for 16 h. Et_2O (10 mL) was added and the organics washed with NaCl (3 × 15 mL, sat. aq.). The organics were dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography (hexane:EtOAc, 100:1) to afford cyclohexenyl amine **5c** as a colourless oil (157 mg, 71%).

R_f [hexane:EtOAc, 3:1] = 0.75; υ_{max} ($CHCl_3$)/cm^{-1}1694 (C=O); ^{1}H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.29-7.22 (2H, m, 2×Ar*H*), 7.01 (1H, dt, *J* 8.4, 2.6, Ar*H*), 5.86-5.84 (1H, m, C*H*=CH), 5.46 (1H, d, *J* 10.2, CH=C*H*), 4.83 (1H, br s, *CHN*), 4.30-4.26 (2H, m, CH_2Ar), 2.06-1.85 (3H, m, CH_2+CH_AH$_B$), 1.79-1.73 (1H, m, CH$_A$$H_B$), 1.70-1.55 (1H, m, C$H_CH_D$), 1.55-1.20 (10H, m, CH$_C$$H_D$+3×C$H_3$); ^{13}C NMR δ (90.6 MHz, 323 K, $CDCl_3$) 161.0 (d, *J* 249.0, C), 155.7 (C), 135.0 (C), 131.4 (CH), 128.5 (CH), 128.0 (CH), 121.7 (d, *J* 9.5, C), 119.4 (d, *J* 24.5, CH), 114.1 (d, *J* 20.9, CH), 79.9 (C), 53.1 (CH), 47.0 (CH_2), 28.2 (3×CH_3), 28.1 (CH_2), 24.5 (CH_2), 21.3 (CH_2); *m/z* (FAB, 3-NOBA) 386 ([^{81Br}M+H]$^+$, 86 %), 384 ([^{79Br}M+H]$^+$, 95), 330 (98), 328 (98), 301 (47), 299 (49), 284 (96), 282 (97), 250 (96), 248 (97), 246 (94), 204 (77), 202 (87); HRMS (FAB, 3-NOBA) Found: [^{79Br}M]$^+$, 384.0969. $C_{18}H_{24}{}^{79}BrFNO_2$ requires 384.0969.

Fluoro phenanthridines 6-8c: To a degassed solution of cyclohexene **5c** (80 mg, 0.21 mmol) in DMF was added the Herrmann-Beller palladacycle (10 mg, 10 µmol) and $MeNCy_2$ (175 µl, 0.84 mmol), and the reaction was heated at 140°C for 5 h. The mixture was allowed to cool and then diluted with Et_2O (20 mL) and washed with NaCl (3 × 20 mL, sat. aq.). The organics were combined, dried ($MgSO_4$) and concentrated under reduced pressure to give the crude product. Flash chromatography (hexane:EtOAc, 100:1-100:6) afforded phenanthridines **6-8c** as a colourless oil (46 mg, 72%). ^{1}H NMR of this oil showed it to be a 27: 44: 29 mixture of double bond isomers (**6c**:**7c**:**8c**).

(4a*SR*,10b*SR)*-9-Fluoro-4,4a,6,10b-tetrahydro-3*H*-phenanthridine-5-carboxylic acid tert-

butyl ester 6c ($\Delta^{1,2}$ isomer): R_f [hexane:EtOAc, 3:1] = 0.57; υ_{max} ($CHCl_3$)/cm^{-1} 1692 (C=O); ^1H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.07 (1H, dd, *J* 8.4, 5.7, Ar*H*), 7.00 (1H, dd, *J* 9.9, 2.6, Ar*H*), 6.88 (1H, td, *J* 8.4, 2.6, Ar*H*), 6.12-6.06 (1H, m, CHC*H*=CH), 5.89-5.87 (1H, m, CH=C*H*CH_2), 4.65 (1H, d, *J* 16.3, C$H_X$$H_Y$Ar), 4.40 (1H, br s, NC*H*CH), 4.36 (1H, d, *J* 16.3, $CH_X$$H_Y$Ar), 3.53 (1H, br s, NCHC*H*), 2.31-2.18 (1H, m, C$H_A$$H_B$), 2.17-2.08 (1H, m, $CH_A$$H_B$), 1.77-1.68 (1H, m, C$H_C$$H_D$), 1.53-1.46 (10H, m, 3×CH_3+$CH_C$$H_D$); ^{13}C NMR δ (90.0 MHz, 323 K, $CDCl_3$) 161.9 (d, *J* 244.1, C), 154.6 (C), 140.0 (d, *J* 6.8, C), 129.0 (CH), 127.5 (d, *J* 8.0, CH), 126.5 (CH), 123.4 (C), 114.4 (d, *J* 22.1, CH), 113.0 (d, *J* 21.8, CH), 79.8 (C), 50.2 (CH), 43.0 (CH_2), 37.3 (CH), 28.5 (3×CH_3), 25.4 (CH_2), 24.0 (CH_2); *m/z* (EI) 303 ($[M]^+$, 8 %), 247 (95), 246 (90), 202 (27), 193 (100), 148 (36); HRMS (EI) Found: $[M]^+$ 303.1626. $C_{18}H_{22}FNO_2$ requires 303.1629. **Diagnostic ^{1}H NMR data for 7c ($\Delta^{2,3}$ isomer)** ^1H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.12 (1H, dd, *J* 8.3, 5.7, Ar*H*), 6.94 (1H, d, *J* 10.5, Ar*H*), 6.92-6.87 (1H, m, Ar*H*), 5.69-5.66 (1H, m, C*H*=CH), 5.46-5.41 (1H, m, CH=C*H*), 4.57 (1H, d, *J* 16.2, C$H_X$$H_Y$), 4.46 (1H, br s, NC*H*), 4.45 (1H, d, *J* 16.1, $CH_X$$H_Y$), 3.18 (1H, s, NCHC*H*), 2.79-2.73 (1H, m, C$H_A$$H_B$), 2.67-2.60 (1H, m, $CH_A$$H_B$), 2.27-2.20 (1H, m, C$H_C$$H_D$), 1.53-1.45 (10H, m, $CH_C$$H_D$+3×C$H_3$). **Diagnostic ^{1}H NMR data for 8c ($\Delta^{3,4}$ isomer)** ^1H NMR δ (360 MHz, 323 K, $CDCl_3$); 5.69-5.66 (1H, m, C*H*=CH), 5.52 (1H, dd, *J* 10.2, 1.0, CH=C*H*), 5.08 (1H, br s, NC*H*), 4.84 (1H, d, *J* 16.4, C$H_X$$H_Y$), 4.19 (1H, d, *J* 16.4, $CH_X$$H_Y$), 3.28 (1H, br s, NCHC*H*), 2.40-2.30 (1H, m, C$H_A$$H_B$), 2.12-2.00 (1H, m, $CH_A$$H_B$).

9-Fluoro-3,4-dihydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylic acid *tert*-butyl ester 17-19c: To a solution of phenanthridines **6-8c** (50 mg, 0.165 mmol) in THF (920 µl) and H_2O (184 µl) was added OsO_4 (144 µl, 2.5% $^w/_w$ in ^{t}BuOH, 11.5 µmol) and NMO (58 mg, 0.495 mmol) and the reaction was stirred for 16 h at r.t. The reaction mixture was poured onto Na_2SO_3 (30 mL, sat. aq.) and extracted with EtOAc (3 × 30 mL). The combined organics were dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2:MeOH, 100:2) to afford a mixture of diols **17-19c** (46 mg, 82%). HPLC (EtOAc:hexane, 3:1) of this mixture afforded $\Delta^{1,2}$ diol **17c** (10 mg, 18%), $\Delta^{2,3}$ diol **18c** (15.3 mg, 2%), $\Delta^{3,4}$ diol **19c** (11.4 mg, 20%), and mixed diol fractions (9 mg, 16%) giving an overall yield (45.7 mg, 82%), all colourless oils.

(1*RS*,2*SR*,4a*SR*,10b*SR*)-9-Fluoro-1,2-dihydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylic acid *tert*-butyl ester 17c ($\Delta^{1,2}$ isomer): R_f [CH_2Cl_2:MeOH, 9:1] = 0.43; R_t (EtOAc:hexane, 3:1, flow rate: 10 mL min^{-1}) = 15 min; υ_{max} ($CHCl_3$)/cm^{-1}3425 (OH), 1664 (C=O); ^1H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.13-7.04 (2H, m, 2×Ar*H*), 6.92 (1H, td, *J* 10.9, 2.6, Ar*H*), 4.70-4.62 (3H, m, 2×C*H*+C$H_X$$H_Y$Ar), 4.31 (1H, d, *J* 16.9, $CH_X$$H_Y$*Ar*), 3.73-3.68 (1H, m, C*H*), 3.37 (1H, br s, C*H*), 2.51 (1H, br s, O*H*), 1.92-1.84 (1H, m, C$H_A$$H_B$), 1.70-1.52 (2H, m, $CH_A$$H_{B+}CH_C$$H_D$), 1.51 (9H, s, 3×C$H_3$), 1.41-1.32 (1H, m, $CH_C$$H_D$); ^{13}C NMR δ (90.6 MHz,

323 K, $CDCl_3$) 161.9 (d, *J* 245.2, C), 154.8 (C), 135.3 (d, *J* 6.9, C), 129.1 (C), 128.1 (d, *J* 8.2, CH), 113.5 (d, *J* 21.7, CH), 112.5 (d, *J* 22.4, CH), 80.1 (C), 71.1 (CH), 67.4 (CH), 46.9 (CH), 43.0 (CH_2), 42.9 (CH), 28.4 ($3\times CH_3$), 27.3 (CH_2), 24.2 (CH_2); *m/z* (EI) 337 ($[M]^+$, 1 %), 280 ($[M-{}^tBu]^+$, 100), 236 ($[M\text{-Boc}]^+$, 61), 192 (19), 162 (26).

(2*RS*,3*SR*,4a*SR*,10b*SR*)-9-Fluoro-2,3-dihydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylic acid *tert*-butyl ester 18c ($\Delta^{2,3}$ isomer): R_f [CH_2Cl_2:MeOH, 9:1] = 0.53; R_t (EtOAc:hexane, 3:1, flow rate: 10 mL min^{-1}) = 20 min; υ_{max} ($CHCl_3$)/cm^{-1} 3421 (OH), 1670 (C=O); ^{1}H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.17 (1H, d, *J* 10.1, Ar*H*), 7.11-7.07 (1H, m, Ar*H*), 6.91 (1H, td, *J* 8.3, 1.8, Ar*H*), 4.80-4.72 (1H, m, C*H*), 4.66 (1H, d, *J* 17.0, CH_XH_YAr), 4.31 (1H, d, *J* 17.0, CH_XH_YAr), 3.93 (1H, br s, C*H*), 3.64-3.60 (1H, m, C*H*), 3.23 (1H, br s, C*H*), 2.40-2.23 (2H, m, CH_2), 1.92-1.89 (1H, m, CH_AH_B), 1.52 (9H, s, $3\times CH_3$), 1.50-1.42 (1H, m, CH_AH_B); ^{13}C NMR δ (62.9 MHz, $CDCl_3$) 164.9 (d, *J* 133.4, C), 154.7 (C), 136.8 (C), 128.6 (C), 127.9 (d, *J* 8.3, CH), 113.4 (d, *J* 21.6, CH), 112.5 (d, *J* 21.9, CH), 80.2 (C), 69.1 (CH), 66.6 (CH), 46.3 (CH), 42.8 (CH_2), 36.4 (CH), 31.3 (CH_2), 29.1 (CH_2), 28.4 ($3\times CH_3$); *m/z* (EI) 337 ($[M]^+$, 1 %), 280 ($[M-{}^tBu])^+$, 100), 236 ($[M\text{-Boc}]^+$, 27), 218 (19), 192 (35), 162 (34), 148 (24).

(3*RS*,4*SR*,4a*RS*,10b*SR*)-9-Fluoro-3,4-dihydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylic acid *tert*-butyl ester 19c ($\Delta^{3,4}$ isomer): R_f [CH_2Cl_2:MeOH, 9:1] = 0.47; R_t (EtOAc:hexane, 3:1, flow rate: 8 mL min^{-1}) = 17 min; υ_{max} ($CHCl_3$)/cm^{-1} 3404 (OH), 1670 (C=O); ^{1}H NMR δ (360 MHz, 323 K, $CDCl_3$) 7.11 (1H, dd, *J* 8.5, 5.7, Ar*H*), 7.03 (1H, dd, *J* 10.0, 1.7, Ar*H*), 6.92 (1H, m, Ar*H*), 4.73-4.61 (2H, m, C*H*+CH_XH_YAr), 4.41 (1H, d, *J* 16.4, CH_XH_YAr), 3.96 (1H, d, *J* 2.7, C*H*), 3.30-3.25 (2H, m, 2×C*H*), 2.60 (1H, br s, O*H*), 2.35-2.22 (1H, m, CH_AH_B), 2.17-2.12 (1H, m, CH_AH_B), 1.87-1.82 (1H, m, CH_CH_D), 1.60-1.50 (10H, m, CH_CH_D+$3\times CH_3$); ^{13}C NMR δ (90.6 MHz, 323 K, $CDCl_3$) 162.1 (d, *J* 245.1, C), 155.2 (C), 137.1 (d, *J* 2.8, C), 128.9 (C), 128.0 (d, *J* 8.2, CH), 113.4 (d, *J* 21.7, CH), 112.3 (d, *J* 22.1, CH), 81.0 (C), 69.5 (2×CH), 52.7 (CH), 42.1 (CH_2), 37.1 (CH), 28.4 ($3\times CH_3$), 25.4 (CH_2), 20.1 (CH_2); *m/z* (EI) 337 ($[M]^+$, 1 %), 281 (36), 280 ($[M-{}^tBu])^+$, 9), 236 ($[M\text{-Boc}]^+$, 100), 218 (11), 206 (12), 192 (13), 162 (40).

(1*RS*,2*SR*,4a*SR*,10b*SR*)-9-Fluoro-1,2,3,4,4a,5,6,10b- octahydro-phenanthridine-1,2-diol 20c ($\Delta^{1,2}$ isomer): To a solution of diol **17c** (10 mg, 30 μmol) in CH_2Cl_2 (2 mL) was added TFA (5 mL) and the reaction was stirred at r.t. for 2 h. The reaction was diluted with H_2O (15 mL), adjusted to pH 8-9 by the addition of NaOH pellets, and then extracted with CH_2Cl_2 (3 × 15 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. The resultant oil was taken up in CH_2Cl_2 (1 mL), cooled to 0°C and HCl (excess, 1 M in Et_2O) added. The resultant oil was washed with Et_2O and dried under vacuum to afford amine hydrochloride

20c as a colourless oil (9 mg, 99%).

^{1}H NMR δ (360 MHz, D_2O) 7.09 (1H, dd, *J* 8.6, 5.7, Ar*H*), 7.03 (1H, dd, *J* 10.2, 2.6, Ar*H*), 6.94 (1H, td, *J* 8.6, 2.6, Ar*H*), 4.28 (1H, d, *J* 16.2, CH_XH$_Y$Ar), 4.22 (1H, d, *J* 16.2, CH$_X$$H_Y$Ar), 3.88 (1H, d, *J* 7.6, C*H*), 3.78-3.74 (2H, m, 2×C*H*), 3.19 (1H, dd, *J* 8.2, 4.4, C*H*), 2.01-1.91 (1H, m, CH_AH$_B$), 1.81-1.72 (1H, m,CH$_A$$H_B$), 1.68-1.60 (2H, m, C$H_2$); ^{13}C NMR δ (90.6 MHz, D_2O) 162.3 (d, *J* 243.8, C), 135.0 (d, *J* 7.7, C), 129.2 (d, *J* 8.5, CH), 123.7 (C), 116.4 (d, *J* 23.7, CH), 115.5 (d, *J* 22.1, CH), 70.9 (CH), 68.2 (CH), 52.0 (CH), 43.4 (CH_2), 39.5 (CH), 25.6 (CH_2), 21.9 (CH_2); *m/z* (ESI+) 238 ([M+H]$^+$, 100 %), 236 (86); HRMS (ESI+) Found [M+H]$^+$, 238.1237. $C_{13}H_{17}O_2NF$ requires 238.1238.

Zebrafish screening: Small breeding tanks were set up in the evening, each containing a male and a female wildtype zebrafish separated by a divider. The tanks were kept in darkness until the next morning when the lights were switched on and the dividers removed, causing the fish to breed. The embryos were collected, pooled and washed with E3 medium. Dead, delayed or unfertilised eggs were discarded, and the adult fish were returned to their main tank. The embryos were stored in an incubator at 28.5°C until required they reached the 'high stage' of development (approxiately 3.3 hpf). Chemicals were diluted into the E3 screening medium.[19] Aliquots of 200 μL were prepared at concentrations ranging from 100 μM to 1 μM, all with 0.5% $^{v}/_{v}$ DMSO. Once the embryos had reached the desired age they were distributed in 96-well plates as appropriate to the screen, with two or three embryos per well. The surrounding medium was removed from the embryos using a wide-tipped Pasteur pipette and then the appropriate 200 μL chemical aliquot was added to the well. Four control wells were used per plate, each containing E3 medium with 0.5% $^{v}/_{v}$ DMSO. The plates were incubated at 28.5°C and examined periodically over the next five days by optical microscope.

Notes and references

[1] (a) D. R. Spring, *Chem. Soc. Rev.* 2005, **34**, 472-482. (b) N. J. Westwood, *Phil. Trans. R. Soc. Lond. A* 2004, **362**, 2761-2774.

[2] (a) F. Landi, C. M. Johansson, D. J. Campopiano and A. N. Hulme, *Org. Biomol. Chem.* 2010, **8**, 56-59. (b) S.-E. Ong, M. Schenone, A. A. Margolin, X. Li, K. Do, M. K. Doud, D. R. Mani, L. Kuai, X. Wang, J. L. Wood, N. J. Tolliday, A. N. Koehler, L. A. Marcaurelle, T. R. Golub, R. J. Gould, S. L. Schreiber and S. A. Carr, *Proc. Natl. Acad. Sci. U. S. A.* 2009, **106**, 4617-4622. (c) B. J. Leslie and P. J. Hergenrother, *Chem. Soc. Rev.* 2008, **37**, 1347-1360.

[3] R. J. Spandl, A. Bender and D. R. Spring, *Org. Biomol. Chem.* 2008, **6**, 1149-1158.

[4] S. Ghosh, A. Nie, J. An, Z. Huang, *Curr. Opin. Chem. Biol.* 2006, **10**, 194-202.

[5] K. S. Lam, *Trends Microbiol*. 2007, **15**, 279-289.

[6] a) N. Unver, *Phytochem Rev.* 2007, 6, 125-135. b) J. R. Lewis, *Nat. Prod. Rep*. 1994, **11**, 329-332.

[7] A. Panzer, A. M. Joubert, P. C. Bianchi, E. Hamel, J. C. Seegers, *Eur. J. Cell. Biol.* 2001, **80**, 111-118.

[8] O. Santana, M. Reina, A. L. Anaya, F. Hernandez, M. E. Izquierdo, A.Gonzalez-Coloma, Zeitschrift fuer Naturforschung, C: *J. Biosci*. 2008, **63**, 639-643.

[9] L. R. Donaldson, D. Haigh, A. N. Hulme, *Tetrahedron* 2008, **64**, 4468-4477.

[10] Z. Jin, *Nat. Prod. Rep.* 2003, **20**, 606–614.

[11] For small molecule screening in zebrafish, see: (a) K. L. Taylor, N. J. Grant, N. D. Temperley and E. E. Patton, *Cell Commun. Signal.* 2010, **8**, 11. (b) J. Berger, P. Currie, *Curr. Med. Chem.* 2007, **14**, 2413-2420. (c) L. I. Zon, R. T. Peterson, *Nat. Rev. Drug Disc.* 2005, **4**, 35-44.

[12] V. Namboodiri, B. Campbell, G. A. Flynn, T. Fujimoto, F. P. Hollinger, M. Bukhtiyarova, E. B. Springman, M. Karpusas, M. *Bioorg. Med. Chem. Lett*. 2005, **15***,* 5274-5279.

[13] In order to obtain characterisation details for the $\Delta^{1,2}$ product, we also performed each cyclisation using our cationic conditions.[9] Diagnostic ^{1}H NMR data was then determined for the $\Delta^{2,3}$ and $\Delta^{3,4}$ phenanthridine isomers by comparison with the data for isolated parent Boc-phenanthridines **6-8a**.[14]

[14] L. R. Donaldson, D. Haigh, A. N. Hulme, *Synlett*, 2009, 1587-1590.

[15] I. Kadas, L. Toke, *J. Heterocyclic Chem*. 1998, **35**, 343-348.

[16] Confirmation that the major product was the *exo*-diol was obtained later in this study when 2D NMR was performed on both the major and minor products from the dihydroxylation of piperonyl phenanthridine **7g**. The major product did not show an nOe correlation between either of the ring junction protons and either of the C*H*OH protons, whereas the minor product (the *endo-syn* diol) did.[17]

[17] L. R. Donaldson, PhD Thesis, The University of Edinburgh, 2009.

[18] Determination of the exact ratio was not possible due to the complex mixture of isomeric products.

[19] R. D. Murphey, L. I. Zon, *Methods* 2006, **39**, 255-261.

[20] C. B. Kimmel, W. W. Ballard, S. R. Kimmel, B. Ullmann and T. F. Schilling, *Dev. Dyn.* 1995, **203**, 253-310.

[21] R. W. King, *Chem. Biol.* 1999, **6**, R327-R333.