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PAPER

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## 3,4'-Linked bis(piperidines) related to the haliclonyclamine class of marine alkaloids: synthesis using crossed-aldol chemistry and preliminary biological evaluations†

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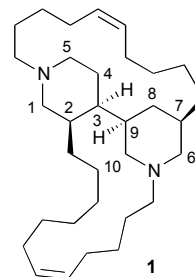
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Compounds 2–5, incorporating various elements of the 3,4'-bis(piperidine) core associated with the sponge-derived alkaloid haliclonyclamine A (HA, **1**), have been prepared through, *inter alia*, aldol-type reactions of *N*-substituted piperidin-4-ones and certain derivatives. Screening of these compounds in various assays, including an ecological one, reveals that compound **5** exhibits allelochemical properties similar to those associated with HA itself.

### Introduction

The natural product haliclonyclamine A (HA, **1**) was first isolated from the sponge *Haliclona* sp. 628 that inhabits the coral reefs adjacent to Heron Island on the Great Barrier Reef. The structure, including absolute configuration, of this novel tetracyclic alkylpiperidine alkaloid was established using a combination of NMR and X-ray crystallographic techniques.<sup>1</sup> Structurally-related alkaloids (halicyclamine A, haliclonyclamines B–F, tetrahydrohaliclonyclamine A, halichondramide, tetrahydrohalicyclamine A) varying, *inter alia*, in the positions and degrees of unsaturation of the hydrocarbon chains linking the 3,4'-bis(piperidine) cores of these systems have been reported.<sup>2</sup> Other structural variants include those incorporating hydroxy groups in the lower chain (arenosclerins A–E, 22-hydroxyhalicyclamine A, 22-hydroxyhaliclonyclamine B),<sup>2a,2e,3</sup> differently sized “spacer groups” (halicyclamine B, neopetrosamine, xestoproxamines A–B)<sup>4,5</sup> and, intriguingly, a methyl-branched spacer group (xestoproxamine C).<sup>5</sup> This extraordinary suite of compounds is probably generated *in vivo* in a manner similar to that proposed by Baldwin and Whitehead for the biogenesis of the manzamine alkaloids<sup>6</sup> and therefore involving, as the pivotal steps, intramolecular Diels–Alder cycloaddition and retro-Mannich-type fragmentation reactions.<sup>1c</sup>

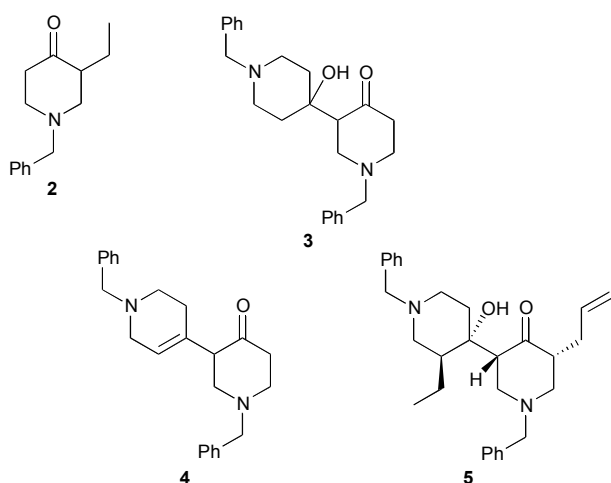
The haliclonyclamines exhibit strong antimicrobial activity and potent cytotoxicity against various cancer cell lines.<sup>1a,1b,2b,2c</sup> Furthermore, haliclonyclamines A and B have recently been shown to exhibit anti-mycobacterial activity against *Mycobacterium smegmatis* and *M. Bovis* Bacille de Calmette et Guérin (BCG) under both aerobic and hypoxic conditions.<sup>3</sup> A study on the mechanism of anti-mycobacterial action of the



related halicyclamine A has been reported.<sup>3b</sup> Semi-synthetically-derived tetrahydrohaliclonyclamine A ( $\equiv$  perhydrohaliclonyclamine<sup>2c</sup>) is cytotoxic,<sup>1a,1b</sup> binds to a variety of ion channels/GPCRs as well as acting as a muscarinic M<sub>1</sub> antagonist, a mode of action that may be related to its cytotoxicity.<sup>7</sup> However, the most intriguing biological activities associated with the haliclonyclamines are their *in situ* effects. Thus, an ecological study revealed that the natural mixture of haliclonyclamines A–D is an effective toxin towards coral tissue and deters feeding by reef fish,<sup>8</sup> while pure haliclonyclamine A exhibits anti-fouling properties because it inhibits the metamorphosis of the larvae of a wide range of taxa, including the ascidian *Herdmania momus* (syn *curvata*).<sup>9</sup>

Despite their fascinating molecular architectures and biological properties, thus far there has been only a handful of reports concerned with the total synthesis of the haliclonyclamine framework.<sup>7</sup> As such the structural features of these natural products responsible for their activities remains uncertain, although the tetra-alkylated 3,4'-bis(piperidine) core is almost certain to be a crucial motif. Herein, therefore, we describe simple synthetic protocols based on, *inter alia*, aldol-type

chemistry that have allowed for the rapid assembly of compounds 2–5 related to the haliclonyclamine core. We also report on the outcomes of the preliminary biological evaluation of these same compounds that has revealed that the most elaborate of them (compound 5) retains much of the activity associated with the parent haliclonyclamine framework.

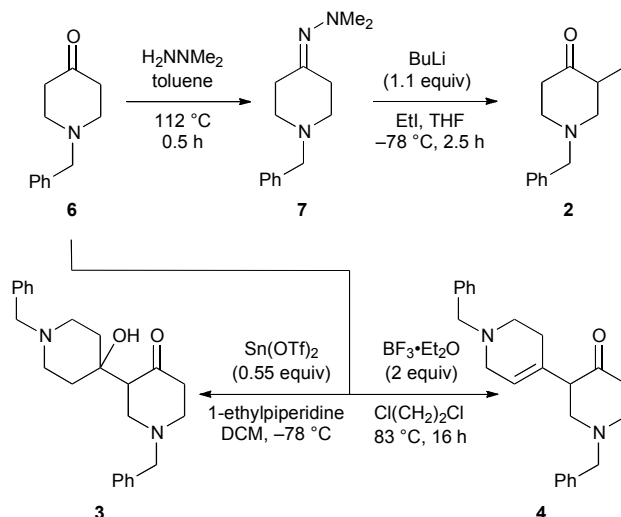


## Results and discussion

### Synthetic studies

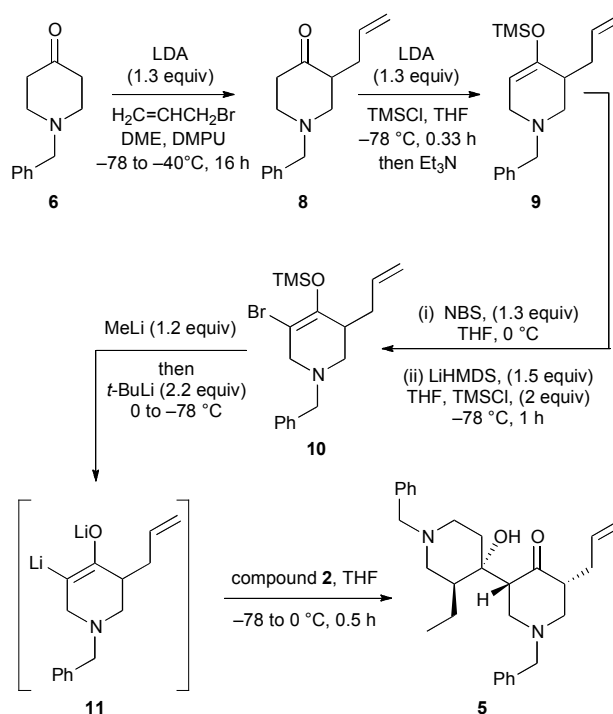
The commercially available 1-benzyl-4-piperidinone (**6**) served as the starting material for the synthetic aspects of the present study and the capacity of various derived enolates to participate in *C*-alkylation reactions, including aldol-type processes, was explored as a means of assembling the targeted substructures 2–5 of HA (**1**). The benzyl group was chosen to protect the secondary amine residue of 4-piperidinone because of its ease of removal with triphosgene.<sup>10</sup> Our first experiments (Scheme 1) rapidly established that the previously unreported but readily prepared *N,N*-dimethylhydrazone (DMH) derivative, **7** (100%), of ketone **6** was smoothly *C*-ethylated upon sequential treatment with BuLi then ethyl iodide.<sup>11</sup> After workup the required ketone **2** was obtained in 81% yield and thereby representing a useful improvement upon a previously reported<sup>12</sup> route to this compound. Two distinct reaction conditions were examined in an effort to effect the self-aldol-type reaction of compound **6** which, if successful, would lead to the 3,4'-bis(piperidine) core of HA. When the tin(II)-mediated aldol process was applied to compound **6** at  $-78\text{ }^{\circ}\text{C}$  then the desired aldol product **3** was obtained in *ca.* 60% yield. In contrast, when the same substrate **6** was subjected to treatment with 2 molar equivalents of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  in refluxing 1,2-dichloroethane then the unsaturated 3,4'-bis(piperidine) **4** was formed, presumably *via* intermediate **3**, in 64% yield. Compound **4** was accompanied by *ca.* 16% of the corresponding conjugated enone from which it could be separated by conventional chromatographic methods.

A necessary requirement for the development of a total synthesis of HA was the identification of protocols that allow for the crossed-aldol reaction of two differentially substituted 4-piperidinones. Since neither of the procedures depicted in Scheme 1 proved effective in this regard, alternatives were



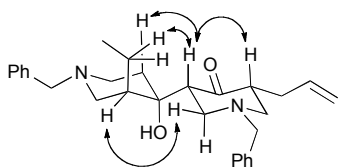
Scheme 1

sought. Ultimately, the use of an  $\alpha$ -keto dianion intermediate<sup>13</sup> provided the desired crossed-aldol product **5** (Scheme 2). Thus, the enolate derived by treating compound **6** with lithium diisopropylamide (LDA) in the presence of DMPU could be trapped with allyl bromide to give compound **8**<sup>14</sup> in 45% yield. Addition of a THF solution of the latter to a mixture of LDA/trimethylsilyl chloride (TMS-Cl)<sup>15</sup> maintained at  $-78\text{ }^{\circ}\text{C}$  resulted in the selective formation of the kinetically favoured silyl enol ether **9** (quantitative yield) which when treated with *N*-bromosuccinimide (NBS) gave the expected  $\alpha$ -allyl- $\alpha'$ -bromoketone as a *ca.* 1:1 mixture of *cis*- and *trans*-isomers. This sensitive ketone was immediately treated with lithium hexamethyldisilazide (LiHMDS) and the resulting enolate trapped with TMS-Cl at  $-78\text{ }^{\circ}\text{C}$  to give the brominated silyl enol ether **10** in 49% yield.



Scheme 2

In the final and pivotal step of the reaction sequence, compound **10** was treated sequentially with methyl lithium then *t*-butyllithium and the  $\alpha$ -keto dianion **11** so formed was trapped with 1-benzyl-3-ethyl-4-piperidinone (**2**) and thereby forming  $\beta$ -hydroxyketone **5** (24%) which could be separated from various co-produced diastereoisomers (44% combined yield) using flash chromatographic techniques. The structure of **5** was established by NMR spectroscopic methods, with nOe techniques being used to determine the illustrated stereochemistry (Fig. 1).



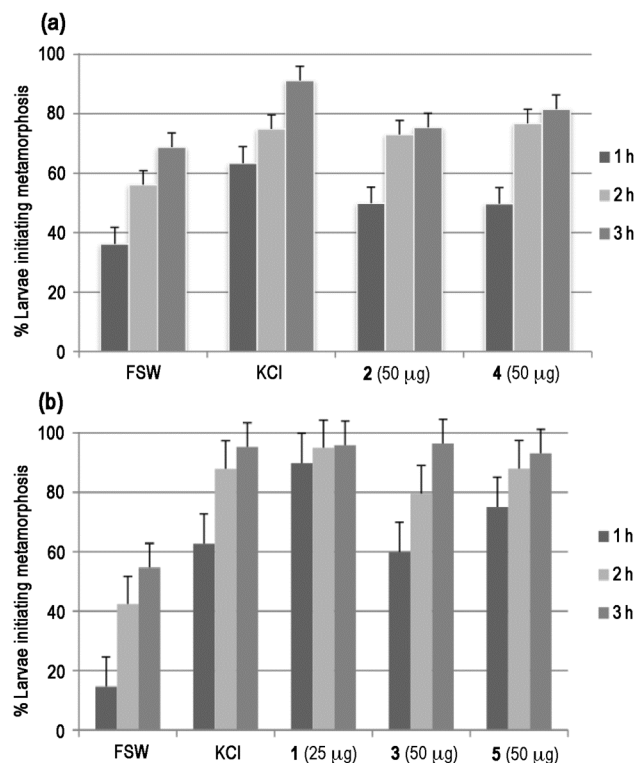
**Fig. 1.** Significant nOe interactions used in assigning the structure of compound **5**.

Each of the reaction sequences presented above serves to highlight the capacity of aldol reactions to generate 3,4'-bis(piperidines) including tetra-alkylated variants. As such, this type of chemistry, when used in conjunction with ring-closing metathesis (RCM) protocols, could provide a means for developing total syntheses of the haliclonyclamines themselves. Work directed towards such ends is now underway in our laboratories.

### Biological evaluation of compounds 2–5

Our previous ecological studies have established that HA (**1**) inhibits the settlement and metamorphosis of sponge, polychaete, gastropod and bryozoan larvae.<sup>9</sup> The synthetic compounds **2–5** were screened against small populations of the larvae of the solitary ascidian *Herdmania momus*, commonly found on the Great Barrier Reef, to test if the various treatments induced or inhibited settlement, and to establish whether larvae subsequently progressed normally through metamorphosis under these conditions. Since there is natural variation in larval settlement rates, the assay runs included experimental controls to assess spontaneous effects in filtered seawater (FSW) or in the presence of the known settlement inducer 40 mM KCl-elevated seawater.<sup>9</sup>

In an initial assay, compounds **2** and **4** were screened at 25  $\mu$ g and at 50  $\mu$ g per well. At 25  $\mu$ g, settlement rates shown by treatment larvae were similar to those in FSW and larvae progressed normally (data not shown). At 50  $\mu$ g per well [Fig. 2(a)], all sets of treated larvae showed initial settlement rates that were elevated above those of FSW, but these were not as pronounced as for KCl-treated larvae; at 3 h, between 70–80% of treatment larvae had settled compared to <70% of the control larvae (FSW). At 12 h, postlarvae exposed to compound **2** showed normal development (characterised by a rounded morphology and the formation of ampullae) while those exposed to compound **4** were retarded and lacked any sign of ampullar development. After 24 h there was a further pronounced difference in the appearance of the two sets of treated postlarvae, while at 36 h those exposed to compound **2** were behind the control larvae in development although still developing normally. In contrast, larvae exposed to compound **4** were necrotic after 36 h.



**Fig. 2.** The effects of haliclonyclamine mimic compounds on settlement of larvae of the Great Barrier Reef ascidian *Herdmania momus*. (a) Data for compounds **2** and **4** (top); (b) Data for compounds **3** and **5** compared to the natural sponge metabolite haliclonyclamine A (HA, **1**) (bottom).

A second assay run compared compounds **3** and **5** at 25, 50 and 100  $\mu$ g per well, and compound **4** at 100  $\mu$ g per well against the inhibitory effects of HA (**1**) at 25  $\mu$ g per well. At 50  $\mu$ g per well for the two mimics, all sets of treated larvae again showed initial settlement rates that were elevated above those of FSW [Fig 2(b)]; at 3 h, between 70–80% of treatment larvae had settled compared to <60% of the control larvae (FSW). However, settlement rates for compound **3** were not as pronounced as for HA (**1**) or compound **5**, or for KCl-treated larvae. The settlement rates for analogue **5** were comparable to those of KCl-treated larvae, but did not show the rapid response associated with the HA treatment. At 3 h, between 70–80% of these treated larvae had initiated metamorphosis whereas close to 90% of HA-treated larvae initiated metamorphosis within 1 h.<sup>9</sup> At 3 h, postlarvae exposed to HA (**1**) showed abnormal development and within 8 h all of these larvae were dead. Those exposed to compounds **3** and **5** were progressing through normal metamorphosis at the 3 h stage, but were mildly retarded compared to control larvae; evidence of abnormal development was apparent after 8 h, and all of these treated larvae were dead after 12 h. For both compounds **3** and **5**, these abnormal developmental effects were less apparent in the 25  $\mu$ g treatment; larvae exposed to compound **3** continued through metamorphosis for 24 h, but showed some abnormalities, while those exposed to compound **5** progressed normally (but behind KCl) for the first 8 h, but were dead at 12 h. At 100  $\mu$ g, larvae exposed to both compounds **3** and **5** were significantly retarded compared to controls, and all larvae were dead at 12 h.

In summary, then, these assays revealed that compound **2** had no marked effect on larval settlement and metamorphosis, while compounds **3–5** each inhibited development, but at a higher concentration than the natural metabolite haliclonyclamine A. The most potent synthetic analogue was compound **5**. In ascidians, haliclonyclamine A inhibits formation of ampullae and other postlarval structures at a key development point in early metamorphosis that corresponds to an increase in gene activity.<sup>9a</sup> These toxic effects benefit the sponge in two ways: firstly, a potential competitor is removed; secondly, the dead postlarvae are a potential source of food for either the sponge or associated microorganisms.

For comparison with their ecological effects, compounds **2–5** were also subjected to cytotoxicity, antimicrobial, and antiparasitic screening. Table 1 shows that when tested at a concentration of 20  $\mu\text{M}$ , compounds **2–5** inhibited cell growth by >10% in 3, 6, 6, and 10 of the 13 different human cancer cell lines targeted. These data reveal that compounds **2** and **5** were, respectively, the least effective and most effective cytostatic compounds, results that parallel the ecological data. Haliclonyclamine A was not screened directly against the thirteen cell-line panel, but earlier screening against the NCI 60 cell line panel had previously revealed its significant cytotoxic effects against certain cancer cell lines<sup>16</sup> and a detailed study of its effects on cultured human cervical cancer (HeLa) cell lines has recently been presented. Thus, HeLa cells exposed to HA at a concentration of 22  $\mu\text{M}$  appeared abnormal within 2 h, a marked change in cell permeability was observed within 4 h, and there was >90% cell death by 24 h.<sup>9b</sup>

**Table 1** Percent inhibition of cell growth<sup>a</sup> caused by 20  $\mu\text{M}$  samples of compounds **2–5** in cytotoxicity assays involving thirteen human cancer cell lines<sup>b</sup>

Cell line <sup>c</sup>	Compound			
	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
A375	–2	10	18	22
A431	3	9	6	23
HCT-15	–2	1	13	77
A549	–4	5	5	4
PA-1	–4	–1	4	12
HT-1376	9	11	11	20
MES-SA-Dx5	16	20	9	19
MCF-7	–9	–5	–1	–2
RAMOS	9	10	9	20
KHOS-NP	2	4	–1	5
BT-20	3	9	11	17
MAT-LyLu	20	20	19	20
DAUDI	18	24	25	61

<sup>a</sup> Percent inhibition values were calculated by comparison with vehicle-treated cells. The numbers shown are calculated. Since cell growth has some inherent variability, the values shown are approximate measurements of inhibition of cellular proliferation, and those identified as <0% simply represent no inhibition of cell growth. <sup>b</sup> DMSO was used as solvent in these assays. <sup>c</sup> See Experimental Section for details.

Screening against a panel of microorganisms (Table 2) revealed that although none of the three synthetic compounds **3**, **4**, or **5** tested was as potent as the natural sponge metabolite, they each possessed moderate activity against *Bacillus subtilis* and the marine fungi *Trichophyton mentagrophytes* and *Cladosporium*

*resinae*. Natural **1** and synthetic **2–5** were also subjected to preliminary screening against the free-living larval stages of the parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*.<sup>17</sup> All five compounds showed modest anthelmintic activity and inhibited larval development by 100% when tested at 100  $\mu\text{g mL}^{-1}$ , while at the lower concentration of 10  $\mu\text{g mL}^{-1}$ , significant numbers of larvae were able to develop as normal for each of the compounds (data not shown). Unfortunately, there was insufficient material for additional screening.

**Table 2** Zones of inhibition (in mm) observed during testing of compounds **1–5** against the microorganisms *E. coli*, *C. albicans*, *B. subtilis*, and the marine fungi *T. mentagrophytes* and *C. resinae* using the Kirby–Bauer or disc diffusion method<sup>d</sup>

Microorganism	Compound				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<i>Escherichia coli</i>	5	ND <sup>b</sup>	– <sup>c</sup>	–	–
<i>Bacillus subtilis</i>	12	ND	3	3	3
<i>Candida albicans</i>	15	ND	–	–	–
<i>Trichophyton mentagrophytes</i>	15	ND	2	5	4
<i>Cladosporium resinae</i>	10	ND	5	2	3

<sup>a</sup> Experiments conducted at 2.0  $\text{g mL}^{-1}$ . <sup>b</sup> ND = not determined. <sup>c</sup> – = no detectable inhibition.

## Conclusions

Although marine ecological studies frequently target structure-activity relationships among naturally-derived candidate allelochemicals,<sup>18</sup> only a few studies have examined the effects of synthetic mimics. Lindel *et al.* assayed fourteen synthetic analogues, in addition to seven bromopyrrole sponge metabolites, for their inhibitory effects on feeding by the omnivorous reef fish *Thalassoma bifasciatum*. They found that a pyrrole group was essential for feeding inhibition activity, while an additional imidazole group further enhanced activity.<sup>19</sup> A subsequent study explored chemical modifications of the pyrrole ring and highlighted the importance of the bromination pattern.<sup>20</sup> Although these and other studies<sup>21</sup> emphasise the key role of individual structural elements in determining ecological effects, the spatial arrangement of functional groups also needs to be considered.<sup>19</sup>

As demonstrated by our recent study on the tambjamins,<sup>22</sup> access to quantities of synthetically-derived metabolites in larger amounts than can be supplied from natural sources significantly extends the capacity for screening against biological targets. Synthetic chemistry also provides rapid access to natural product libraries for compound screening.<sup>23</sup> Our synthetic study provided a suite of mimics of the haliclonyclamine A skeleton that were then screened against a range of biological targets. The low activity noted for the simple 1-benzylpiperidinone (**2**) in the various assays highlights the likely importance of a 3,4'-bis(piperidine) core for effective ecological activity. The synthetic route employed provided a concise route to mimics with this scaffold. Three synthetic compounds that embodied the heterocyclic core of the haliclonyclamines were tested, and all showed useful activity in the larval assays, as well as some antimicrobial activity. Assessment of the antiparasitic activity of the mimics was limited by compound availability. The most potent synthetic compound **5** is a very interesting mimic of the structurally complex and biologically potent haliclonyclamine

A (**1**), as it is the only synthetic compound that replicates a key structural feature of the alkaloid, namely the alkyl substitution at C-2 and at C-7 of **1** that is a natural structural consequence of a biosynthetic pathway involving a nicotinic acid precursor.<sup>1c</sup> The size and shape of alkyl substituents at these positions may well have a profound influence on ecological activity. Accordingly, the screening of more advanced synthetic intermediates would appear to be worthwhile.

## Experimental

### (i) Synthetic studies

#### (a) General experimental protocols

Proton (<sup>1</sup>H) and carbon (<sup>13</sup>C) NMR spectra were recorded on Varian machines operating at 300 or 400 MHz. Unless otherwise specified, the spectra were recorded at 20 °C in deuteriochloroform. Chemical shifts are recorded as δ values in parts per million (ppm). Infrared spectra ( $\nu_{\max}$ ) were recorded as thin films on NaCl plates using a Perkin–Elmer 1800 Series FTIR spectrometer. Low-resolution ESI mass spectra were recorded on a Micromass-Waters LC-ZMD single quadrupole liquid chromatograph mass spectrometer while low and high resolution EI mass spectra were recorded on a VG Fisons AUTOSPEC three-sector double focussing instrument. Flash chromatographic separations were carried out using the protocols defined by Still *et al.*<sup>24</sup> with silica gel 60 (40–63 μm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Melting points were measured using a Reichert hot stage microscope and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminium-backed 0.2 mm thick silica gel 60 F254 plates as supplied by Merck. Eluted plates were visualised using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. The retardation factor ( $R_f$ ) values cited here have been rounded at the first decimal point. Starting materials and reagents were generally available from the Sigma–Aldrich, Merck, TCI, Strem or Lancaster Chemical companies and were used as supplied. Solvents were dried according to the procedures specified by Armarego and Chai.<sup>25</sup> All the reactions were performed under an inert atmosphere of nitrogen.

#### (b) Specific experimental procedures

**Compound 7.** A magnetically stirred solution of 1-benzyl-4-piperidinone (**6**) (1.89 g, 10.0 mmol, *ex.* Aldrich Chemical Co.) in dry toluene (10 mL) maintained at 18 °C was treated with 1,1-dimethylhydrazine (840 μL, 11 mmol, 1.1 mole equiv). The resulting mixture was heated at reflux with azeotropic removal of water (Dean–Stark trap) for 0.5 h then cooled and concentrated under reduced pressure to give *compound 7* (2.31 g, quant.) as a clear, yellow oil. <sup>1</sup>H NMR (300 MHz) δ 7.35–7.25 (5H, complex m), 3.54 (2H, s), 2.68–2.51 (6H, complex m), 2.44 (6H, s), 2.39 (2H, t,  $J = 5.9$  Hz); <sup>13</sup>C NMR (75 MHz) δ 169.9 (C), 138.2 (C), 128.9 (CH), 128.2 (CH), 127.1 (CH), 62.6 (CH<sub>2</sub>), 54.0 (CH<sub>2</sub>), 53.0 (CH<sub>2</sub>), 47.6 (CH<sub>3</sub>), 35.3 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>); IR (NaCl, film)  $\nu_{\max}$  2950, 2904, 2854, 2813, 1641, 1494, 1467, 1453, 1349, 1289, 1199, 1124 cm<sup>-1</sup>; MS (EI)  $m/z$  231 (M<sup>+</sup>, 93%), 216 (43), 187 (28), 186 (29), 173 (12), 146 (53), 97 (56), 91 (100); HRMS calcd for C<sub>14</sub>H<sub>21</sub>N<sub>3</sub> (M<sup>+</sup>) 231.1735. Found 231.1733.

**Compound 2.** A magnetically stirred solution of hydrazone 7

(2.31 g, 10.0 mmol) in dry THF (20 mL) maintained at 0 °C was treated with *n*-BuLi (8.5 mL of a 1.29 M solution in hexanes, 11 mmol, 1.1 equiv). After 0.16 h the reaction mixture was cooled to –78 °C and iodoethane (1.6 mL, 20 mmol, 2.0 equiv) was added. After a further 2.5 h (at –78 °C) the reaction mixture was quenched by the slow addition of HCl (30 mL of a 1 M aqueous solution). The resulting mixture was stirred at 18 °C for 1 h, cooled to 0 °C, basified [with NaOH (*ca.* 15 mL of a 2 M aqueous solution)] to pH 9 then diluted with water (50 mL) and diethyl ether (50 mL). The separated aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL) and the combined organic phases washed with brine (1 × 20 mL) before being dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and then concentrated under reduced pressure to afford a clear, colourless oil. Subjection of this material to flash chromatography (silica, 1:4 → 3:7 v/v ethyl acetate/hexane gradient elution) provided, after concentration of the appropriate fractions ( $R_f = 0.5$  in ethyl acetate), compound **2**<sup>12</sup> (1.76 g, 81%) as a clear, colourless oil. <sup>1</sup>H NMR (300 MHz) δ 7.36–7.21 (5H, complex m), 3.63 (1H, d,  $J = 13.2$  Hz), 3.52 (1H, d,  $J = 13.2$  Hz), 3.00 (1H, ddd,  $J = 11.0, 5.3$  and  $2.2$  Hz), 2.92 (1H, m), 2.55–2.31 (4H, complex m), 2.21 (1H, dd,  $J = 11.0$  and  $9.5$  Hz), 1.81 (1H, m), 1.27 (1H, m), 0.86 (3H, t,  $J = 7.5$  Hz); <sup>13</sup>C NMR (75 MHz) δ 210.5 (C), 138.0 (C), 128.6 (CH), 128.1 (CH), 127.0 (CH), 61.8 (CH<sub>2</sub>), 58.4 (CH<sub>2</sub>), 53.5 (CH<sub>2</sub>), 51.3 (CH), 40.9 (CH<sub>2</sub>), 20.7 (CH<sub>2</sub>), 11.8 (CH<sub>3</sub>); IR (NaCl, film)  $\nu_{\max}$  3028, 2962, 2875, 2800, 2761, 1715, 1602, 1495, 1454, 1356, 1326, 1193, 1135, 1065, 1028, 1002, 867 cm<sup>-1</sup>; MS (EI)  $m/z$  217 (M<sup>+</sup>, 18%), 216 (13), 202 (19), 126 (9), 91 (100); HRMS calcd for C<sub>14</sub>H<sub>19</sub>NO (M<sup>+</sup>) 217.1467. Found 217.1467.

**Compound 3.** A magnetically stirred suspension of tin(II) triflate (459 mg, 1.10 mmol, 0.55 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL) maintained at 0 °C was treated with 1-ethylpiperidine (179 μL, 1.30 mmol, 0.65 equiv). The resulting mixture was cooled to –78 °C and a solution of 1-benzyl-4-piperidinone (**6**) (379 mg, 2.00 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added *via* cannula. After 1 h at –78 °C the reaction mixture was slowly warmed to –30 °C and held at this temperature for 2 h. The reaction mixture was then treated with sodium potassium tartrate (5 mL of a 1 M aqueous solution) and CHCl<sub>3</sub> (5 mL) and the ensuing mixture stirred vigorously at 18 °C for 1 h. The phases were separated and the aqueous one was extracted with CHCl<sub>3</sub> (3 × 5 mL). The combined organic phases were washed with brine (1 × 5 mL) before being dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The resulting clear, colourless oil was subjected to flash chromatography (silica, ethyl acetate elution) and concentration of the appropriate fractions ( $R_f = 0.1$ ) gave *compound 3* (230 mg, 61%) as a clear, colourless oil and containing *ca.* 5% of the conjugated isomer of compound **4**. <sup>1</sup>H NMR (500 MHz) δ 7.36–7.21 (10H, complex m), 5.70 (1H, broad s), 3.64 (1H, d,  $J = 12.7$  Hz), 3.53–3.45 (3H, complex m), 3.22 (1H, dt,  $J = 12.2$  and  $2.9$  Hz), 3.05 (1H, ddd,  $J = 12.8, 6.0$  and  $2.9$  Hz), 2.83 (1H, ddd,  $J = 17.6, 10.7$  and  $6.8$  Hz), 2.64–2.50 (4H, complex m), 2.45 (1H, m), 2.40–2.28 (2H, complex m), 2.24 (1H, broad s), 1.60 (1H, ddd,  $J = 13.2, 12.2$  and  $4.4$  Hz), 1.47 (1H, m), 1.44 (1H, m), 1.35 (1H, td,  $J = 11.7$  and  $4.4$  Hz); <sup>13</sup>C NMR (125 MHz) δ 210.0 (C), 138.6 (C), 136.9 (C), 129.2 (CH), 129.1 (CH), 128.6 (CH), 128.2 (CH), 127.9 (CH), 126.9 (CH), 70.3 (C), 63.1 (CH<sub>2</sub>), 62.1 (CH<sub>2</sub>), 58.0 (CH), 53.7 (CH<sub>2</sub>), 53.2 (CH<sub>2</sub>), 49.2

(CH<sub>2</sub>), 49.0 (CH<sub>2</sub>), 41.8 (CH<sub>2</sub>), 36.5 (CH<sub>2</sub>), 36.1 (CH<sub>2</sub>); IR (NaCl, film)  $\nu_{\max}$  3270, 3028, 2941, 2815, 1707, 1453, 1345, 1120, 1027, 998, 909, 812 cm<sup>-1</sup>; MS (EI)  $m/z$  378 (M<sup>+</sup>, 5%), 360 (5), 287 (8), 269 (8), 191 (73), 91 (100); HRMS calcd for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> (M<sup>+</sup>) 378.2307. Found 378.2300.

When the procedure described above was followed except for allowing the reaction mixture to stand to 18 °C for 2 h then a ca. 1:4 mixture of compound **4** and its conjugated isomer (238 mg, 66% combined yield) was obtained.

**Compound 4.** A magnetically stirred solution of 1-benzyl-4-piperidinone (**6**) (8.5 mL, 45.9 mmol, ex. Aldrich Chemical Co.) in dry 1,2-dichloroethane (15 mL) maintained at 40 °C was treated with BF<sub>3</sub>•OEt<sub>2</sub> (11.6 mL, 91.5 mmol, 2 mole equiv). The resulting mixture was heated at reflux for 16 h (during the course of which a viscous brown gum formed) then cooled to 18 °C and poured into NaHCO<sub>3</sub> (200 mL of a saturated aqueous solution). The residual gum was treated with MeOH (30 mL) and the mixture heated at reflux for 0.5 h and the ensuing methanolic solution then cooled to 18 °C before being poured into the NaHCO<sub>3</sub> (200 mL of a saturated aqueous solution). Water (50 mL) and CHCl<sub>3</sub> (100 mL) were added and the phases were separated. The aqueous phase was extracted with CHCl<sub>3</sub> (3 × 50 mL), the combined organic portions were washed with NaHCO<sub>3</sub> (1 × 50 mL of a saturated aqueous solution), water (1 × 50 mL) and brine (1 × 50 mL) before being dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and then concentrated under reduced pressure to afford a brown oil. Subjection of this material to flash chromatography (silica, 3:2 → 4:1 v/v ethyl acetate/hexane gradient elution) provided two fractions, A and B.

Concentration of fraction A ( $R_f$  = 0.25 in 3:2 v/v ethyl acetate/hexane) afforded *compound 4* (5.30 g, 64%) as a clear, yellow oil. <sup>1</sup>H NMR (500 MHz)  $\delta$  7.35–7.24 (10H, complex m), 5.56 (1H, broad s), 3.60 (2H, AB<sub>q</sub>), 3.58 (2H, AB<sub>q</sub>), 3.12 (1H, broad dd,  $J$  = 9.3 and 5.2 Hz), 3.01 (2H, dd,  $J$  = 5.2 and 2.2 Hz), 2.98 (1H, m), 2.94 (1H, m), 2.78 (1H, m), 2.62 (1H, dd,  $J$  = 11.3 and 9.2 Hz), 2.58–2.42 (4H, complex m), 2.08 (2H, m); <sup>13</sup>C NMR (75 MHz)  $\delta$  208.6 (C), 138.1 (C), 138.0 (C), 132.6 (CH), 129.2 (CH), 128.9 (CH), 128.3 (CH), 128.2 (CH), 127.3 (CH), 127.0 (CH), 123.1 (C), 62.8 (CH<sub>2</sub>), 62.2 (CH<sub>2</sub>), 57.1 (CH<sub>2</sub>), 56.7 (CH), 53.6 (CH<sub>2</sub>), 52.9 (CH<sub>2</sub>), 49.6 (CH<sub>2</sub>), 40.9 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>); IR (NaCl, film)  $\nu_{\max}$  3026, 2908, 2800, 2759, 1715, 1493, 1453, 1364, 1349, 1186, 1123, 1027 cm<sup>-1</sup>; MS (EI)  $m/z$  360 (M<sup>+</sup>, 19%), 325 (5), 269 (13), 241 (40), 199 (36), 185 (10), 172 (15), 134 (11), 91 (100); HRMS calcd for C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O (M<sup>+</sup>) 360.2202. Found 360.2201.

Concentration of fraction B ( $R_f$  = 0.15 in 1:99 v/v ammonia/ethyl acetate) afforded *1,1'-dibenzyl-2',3',5',5',6,6'-hexahydro-1H,1'H[3,4'-bipyridinylidene]-4(2H)-one* (the conjugated isomer of compound **4**) (1.36 g, 16%) as a clear, yellow oil. <sup>1</sup>H NMR (300 MHz)  $\delta$  7.36–7.23 (10H, complex m), 3.65 (2H, s), 3.51 (2H, s), 3.39 (2H, broad s), 2.80 (2H, m), 2.78 (2H, m), 2.55 (2H, app t,  $J$  = 6.3 Hz), 2.49 (4H, m), 2.22 (2H, app t,  $J$  = 5.7 Hz); <sup>13</sup>C NMR (75 MHz)  $\delta$  201.6 (C), 147.2 (C), 138.1 (C), 138.0 (C), 129.1 (CH), 128.8 (CH), 128.4 (CH), 128.2 (CH), 127.7 (C), 127.3 (CH), 127.0 (CH), 62.8 (CH<sub>2</sub>), 62.0 (CH<sub>2</sub>), 55.7 (CH<sub>2</sub>), 54.7 (CH<sub>2</sub>), 53.9 (CH<sub>2</sub>), 51.1 (CH<sub>2</sub>), 41.5 (CH<sub>2</sub>), 30.7 (CH<sub>2</sub>), 30.1 (CH<sub>2</sub>); IR (NaCl, film)  $\nu_{\max}$  3027, 2904, 2800, 2757, 1682, 1602, 1494, 1453, 1364, 1347, 1285, 1191, 1126, 1027, 1004 cm<sup>-1</sup>; MS

(EI)  $m/z$  360 (M<sup>+</sup>, 51%), 332 (6), 269 (45), 241 (37), 198 (42), 134 (52), 91 (100); HRMS calcd for C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O (M<sup>+</sup>) 360.2202. Found 360.2199.

**Compound 8.** A magnetically stirred solution of diisopropylamine (3.9 mL, 28 mmol, 1.4 equiv) in dry DME (100 mL) maintained at 0 °C was treated with *n*-BuLi (10.8 mL of a 2.4 M solution in hexanes, 26 mmol, 1.3 mole equiv). After stirring for 0.08 h the reaction mixture was cooled to –78 °C and 1-benzyl-4-piperidinone (**6**) (3.79 g, 20.0 mmol, ex. Aldrich Chemical Co.) was added dropwise. Dry DMPU (4.8 mL, 40 mmol, 2 equiv) was then added to aid solubility. After 0.16 h at –78 °C the reaction mixture was treated with allyl bromide (2.6 mL, 30 mmol, 1.5 mole equiv) then slowly warmed to –40 °C and after 0.5 h at this temperature quenched with NaHCO<sub>3</sub> solution (100 mL of a saturated aqueous solution). The resulting mixture was warmed to 18 °C then diluted with water (100 mL) and diethyl ether (50 mL). The separated aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL) and the combined organic phases washed with brine (1 × 50 mL) before being dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and then concentrated under reduced pressure to give a clear, colourless oil. Subjection of this material to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) provided, after concentration of the appropriate fractions ( $R_f$  = 0.6), compound **8**<sup>14</sup> (2.05 g, 45%) as a clear, colourless oil. <sup>1</sup>H NMR (300 MHz)  $\delta$  7.36–7.25 (5H, complex m), 5.73 (1H, m), 5.05–4.96 (2H, complex m), 3.68 (1H, d,  $J$  = 13.1 Hz), 3.54 (1H, d,  $J$  = 13.1 Hz), 3.10–2.96 (2H, complex m), 2.66–2.35 (5H, complex m), 2.24 (1H, dd,  $J$  = 11.2 and 9.7 Hz), 2.05 (1H, m); <sup>13</sup>C NMR (75 MHz)  $\delta$  210.1 (C), 138.2 (C), 135.7 (CH), 128.9 (CH), 128.4 (CH), 127.3 (CH), 116.7 (CH<sub>2</sub>), 62.0 (CH<sub>2</sub>), 58.6 (CH<sub>2</sub>), 53.6 (CH<sub>2</sub>), 49.4 (CH), 41.1 (CH<sub>2</sub>), 32.0 (CH<sub>2</sub>); IR (NaCl, film)  $\nu_{\max}$  3064, 3028, 2911, 2801, 1715, 1640, 1453, 1354, 1188, 1133, 1028, 999, 915 cm<sup>-1</sup>; MS (EI)  $m/z$  229 (M<sup>+</sup>, 26%), 228 (16), 214 (8), 200 (27), 187 (35), 172 (6), 152 (10), 146 (927), 91 (100); HRMS calcd for C<sub>15</sub>H<sub>19</sub>NO (M<sup>+</sup>) 229.1467. Found 229.1466.

**Compound 9.** A magnetically stirred solution of diisopropylamine (1.1 mL, 7.8 mmol, 1.6 equiv) in dry THF (100 mL) maintained at 0 °C was treated *n*-BuLi (3.1 mL of a 2.4 M solution in hexanes, 7.4 mmol, 1.5 mole equiv). After stirring for 0.08 h the reaction mixture was cooled to –78 °C and TMS-Cl (1.3 mL, 10 mmol, 2 mole equiv) was added dropwise. A solution of ketone **8** (3.79 g, 20.0 mmol) in THF (8 mL) was then added *via* cannula over 0.8 h and the resulting mixture stirred at –78 °C for 0.33 h. Triethylamine (0.5 mL) was then added to the mixture, followed by NaHCO<sub>3</sub> (10 mL of a saturated aqueous) and the resulting mixture warmed to 18 °C before being diluted with water (10 mL) and hexane (10 mL). The separated aqueous phase was extracted with hexane (2 × 10 mL) and the combined organic phases were then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to afford *compound 9* (1.51 g, quant.) as a clear, colourless but unstable oil. <sup>1</sup>H NMR (300 MHz)  $\delta$  7.36–7.22 (5H, complex m), 5.69 (1H, m), 4.92–4.85 (2H, complex m), 4.76 (1H, broad t,  $J$  = ca. 3 Hz), 3.64 (1H, d,  $J$  = 13.0 Hz), 3.46 (1H, d,  $J$  = 13.0 Hz), 3.02 (1H, ddd,  $J$  = 14.8, 3.7 and 1.2 Hz), 2.91 (1H, ddd,  $J$  = 14.8, 3.5 and 1.1 Hz), 2.53–2.34 (3H, complex m), 2.16 (2H, m), 0.21 (9H, s).

**Compound 10.** *Step (i):* A magnetically stirred solution of

compound **9** (1.51 g, 5.00 mmol) in dry THF (25 mL) maintained at 0 °C was treated with NBS (1.16 g, 6.50 mmol, 1.3 mole equiv). After stirring for a further 0.5 h at 0 °C the reaction mixture was treated successively with NaHCO<sub>3</sub> (10 mL of a saturated aqueous solution), sodium thiosulfate (10 mL of a 1 M aqueous solution), water (50 mL) and hexane (50 mL). The separated aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL) and the combined organic ones were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and then concentrated under reduced pressure to afford a yellow oil. Subjection of this material to flash chromatography (silica, 0.2:5:95 → 0.2:15:85 v/v/v triethylamine/ethyl acetate/hexane gradient elution) provided, after concentration of the appropriate fractions ( $R_f = 0.4$  and  $0.3$  in 0.2:15:85 v/v/v triethylamine/ethyl acetate/hexane), the expected mixture of *cis*- and *trans*- $\alpha$ -bromoketones (672 mg, 44%) as a clear, yellow oil. Since this material was found to decompose readily upon standing, it was used immediately in the next step of the sequence.

*Step (ii)*: A magnetically stirred solution of hexamethyl-disilazane (338 mL, 1.6 mmol, 1.6 mole equiv) in dry THF (5 mL) maintained at 0 °C was treated with *n*-BuLi (625 mL of a 2.4 M solution in hexanes, 1.50 mmol, 1.5 equiv). After stirring for 0.08 h the ensuing mixture was cooled to -78 °C and TMS-Cl (254 mL, 2.0 mmol, 2 mole equiv) added dropwise. A solution of the previously prepared mixture of  $\alpha$ -bromoketones (308 mg, 1.00 mmol) in THF (3 mL) was added dropwise, *via* cannula, and the ensuing mixture stirred at -78 °C for 1 h then treated with triethylamine (0.2 mL) and NaHCO<sub>3</sub> (5 mL of a saturated aqueous solution) before being warmed to 18 °C and diluted with water (5 mL) then hexane (5 mL). The separated aqueous phase was extracted with hexane (2 × 5 mL) and the combined organic phases were then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to afford a clear, colourless oil. Subjection of this material to flash chromatography (silica, 0.2:5:95 → 0.2:15:85 v/v/v triethylamine/ethyl acetate/hexane gradient elution) provided, after concentration of the appropriate fractions ( $R_f = 0.6$  in 0.2:15:85 v/v/v triethylamine/ethyl acetate/hexane), compound **10** (185 mg, 49%) as a clear, colourless but highly unstable oil. <sup>1</sup>H NMR (300 MHz)  $\delta$  7.33–7.25 (5H, complex m), 5.63 (1H, m), 4.94–4.85 (2H, complex m), 3.66 (1H, d,  $J = 13.0$  Hz), 3.49 (1H, d,  $J = 13.0$  Hz), 3.34 (1H, broad d,  $J = ca. 14$  Hz), 3.14 (1H, broad d,  $J = ca. 14$  Hz), 2.62–2.48 (2H, complex m), 2.42 (1H, m), 2.29–2.16 (2H, complex m), 0.28 (9H, s).

**Compound 5**. A magnetically stirred solution of compound **10** (180 mg, 0.47 mmol) in dry THF (2.5 mL) maintained at -78 °C was treated with MeLi (1.58 mL of a 0.36 M solution in diethyl ether, 0.57 mmol, 1.2 equiv) and the resulting mixture warmed to 0 °C and stirred at this temperature for 0.75 h. The solution thus obtained was cooled to -78 °C, *t*-BuLi (720  $\mu$ L of a 1.45 M solution in pentane, 1.04 mmol, 2.2 equiv) was added dropwise and the resulting yellow solution was warmed to 0 °C and stirred at this temperature for 0.5 h. The mixture was then recooled to -78 °C and ketone **2** (154 mg, 0.71 mmol, 1.8 equiv) in THF (1.5 mL) was added dropwise *via* cannula and the reaction mixture then stirred at -78 °C for 0.16 h before being warmed to 0 °C. After 0.25 h at 0 °C, pH 7 buffer solution (2 mL) was added, followed by water (5 mL) and hexane (5 mL). The layers were separated and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL). The combined organic portions were dried (Na<sub>2</sub>SO<sub>4</sub>),

filtered and then concentrated under reduced pressure to afford a yellow oil. Subjection of this material to flash chromatography (silica, 1:4 → 99:1 v/v ethyl acetate/hexane gradient elution) provided three fractions, A, B and C.

Concentration of fraction A ( $R_f = 0.5$  in ethyl acetate) provided a *ca.* 1:1 mixture of compounds **2** and **8** (57.1 mg) as a clear, colourless oil.

Concentration of fraction B ( $R_f = 0.3$  in ethyl acetate) provided a clear, yellow oil (93.5 mg) tentatively identified as a mixture of the various diastereoisomers of compound **5** (44%). The various components of this mixture could not be separated from one another by conventional chromatographic methods.

Concentration of fraction C ( $R_f = 0.2$  in ethyl acetate) provided compound **5** (51 mg, 24%) as a clear, yellow oil. <sup>1</sup>H NMR (600 MHz)  $\delta$  7.35–7.22 (10H, complex m), 5.70 (1H, m), 5.02–4.95 (2H, complex m), 3.93 (1H, broad s), 3.63 (2H, AB<sub>q</sub>), 3.57 (1H, d,  $J = 13.1$  Hz), 3.42 (1H, d,  $J = 13.1$  Hz), 3.27 (1H, ddd,  $J = 11.1, 5.3$  and  $2.8$  Hz), 3.17 (1H, ddd,  $J = 11.3, 6.0$  and  $2.8$  Hz), 2.96 (1H, dd,  $J = 11.6$  and  $5.3$  Hz), 2.65 (1H, m), 2.60 (1H, broad d,  $J = ca. 10$  Hz), 2.51 (1H, partially obsc. m), 2.49 (1H, partially obsc. m), 2.35 (1H, broad t,  $J = 11.1$  Hz), 2.31 (1H, app. t,  $J = ca. 11$  Hz), 2.14 (1H, broad t,  $J = ca. 10$  Hz), 2.11 (1H, t,  $J = 11.3$  Hz), 1.92 (1H, m), 1.86 (1H, m), 1.67 (1H, m), 1.51 (1H, broad d,  $J = 13.2$  Hz), 1.34 (1H, m), 1.20 (1H, m), 0.78 (3H, t,  $J = 7.5$  Hz); <sup>13</sup>C NMR (75 MHz)  $\delta$  212.9 (C), 138.6 (C), 137.8 (C), 135.6 (CH), 129.1 (CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 127.4 (CH), 126.9 (CH), 116.7 (CH<sub>2</sub>), 73.0 (C), 63.1 (CH<sub>2</sub>), 61.8 (CH<sub>2</sub>), 59.4 (CH<sub>2</sub>), 55.6 (CH), 55.5 (CH<sub>2</sub>), 53.0 (CH<sub>2</sub>), 49.7 (CH), 48.9 (CH<sub>2</sub>), 42.9 (CH), 35.6 (CH<sub>2</sub>), 31.1 (CH<sub>2</sub>), 20.5 (CH<sub>2</sub>), 12.3 (CH<sub>3</sub>); IR (NaCl, film)  $\nu_{max}$  3478, 3027, 2931, 2806, 1713, 1638, 1494, 1453, 1359, 1138, 1028, 914, 737 cm<sup>-1</sup>; MS (EI)  $m/z$  447 [(M+H)<sup>+</sup>, 19%], 328 (7), 230 (100), 218 (29), 119 (53); HRMS calcd for C<sub>29</sub>H<sub>38</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 447.3012. Found 447.3013.

## (ii) Biological studies

### (a) Ecological assays

Adult specimens of *Herdmania momus* were collected from Heron Island Reef, Great Barrier Reef, Australia, and maintained in flowing seawater at ambient temperatures at Heron Island Research Station. Eggs and sperm dissected out from three *H. momus* individuals were combined in 0.2  $\mu$ M-filtered seawater (FSW) at 23 °C.<sup>9,10</sup> Tailbud embryos were sorted in batches of 30 into 6-well plates, each containing 5 mL of FSW, and immediately after hatching were exposed to treatments as follows: Run 1: compounds **2** and **4** were dissolved in DCM at 25 or 50  $\mu$ g per 10  $\mu$ L; Run 2: haliclonacyclamine A at 25  $\mu$ g per 10  $\mu$ L, plus compounds **3** and **5** at 25, 50 or 100  $\mu$ g per 10  $\mu$ L. Ten microlitres of each concentration was pipetted onto 5 mm Whatman filter paper discs that were briefly dried, and then placed in each well at time  $t = 0$  h, while 10  $\mu$ L of DCM was applied to a filter disc, that was then also briefly dried, to provide a solvent control for each run. In parallel, larvae were cultured in FSW or in 40 mM-elevated KCl (settlement inducer). In each of the two experimental runs, the treatments were carried out in triplicate using larvae reared from the same fertilisation. Numbers of larvae responding to the treatment were scored at hourly intervals from  $t = 0$  h. Larvae were scored as having initiated metamorphosis if 50% tail resorption had been observed.<sup>26</sup>

Larvae were monitored for a 36 h period post-treatment to evaluate longer term effects of individual treatments on larvae. Larvae exposed to filter discs treated with solvent alone showed settlement rates that were comparable to those observed in FSW alone.

### (b) Cytotoxicity testing protocols<sup>22</sup>

Stock solutions of the test compounds were prepared at 20 mM in neat DMSO and diluted in tissue culture medium to a concentration of 20  $\mu$ M. Compounds were screened against ATCC cell lines at a final concentration of 2  $\mu$ M in flat-bottom TC plates. The cells were incubated for 72 h at 37 °C in a 5% CO<sub>2</sub> incubator. Inhibition of cell proliferation by the test compounds was determined by measuring conversion of Alamar-Blue fluorescent dye at excitation/emission 544/590 nm with a BMG FluoStar plate reader. The ATCC cell lines used were: A375 (CRL-1619), Human malignant melanoma; A431 (CRL-1555), Human epithelial carcinoma; HCT-15 (CCL-225), Human colon adenocarcinoma; A549 (CCL-185), Human lung carcinoma; PA-1 (CRL-1572), Human ovarian teratocarcinoma; HT-1376 (CRL-1472), Human bladder carcinoma; MES-SA-Dx5 (CRL-1977), Human uterine sarcoma; MCF-7 (HTB-22), Human breast adenocarcinoma; RAMOS (CRL-1596), Human Burkitt's lymphoma; KHOS-NP (CRL-1544), Human osteosarcoma; BT-20 (HTB-19), Human mammary carcinoma; MAT-LyLu (CRL-2376), Rat prostate carcinoma; DAUDI (CCL-213), Human B lymphoma.

### (c) Studies of antimicrobial effects

Susceptibility tests were performed by the disc diffusion method of Bauer and Kirby<sup>27</sup> using Mueller–Hinton agar (Difco) plates. After inoculation of the plates with ca. 10<sup>6</sup> cells of the relevant micro-organism (*Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Trichophyton mentagrophytes*, *Cladosporium resinae*) paper discs (6 mm  $\times$  0.5 mm) impregnated with a 40  $\mu$ L aliquot of a 2 mg mL<sup>-1</sup> solution of compounds **1**, **3–5** in solvent were placed on the surface of the plate. The plates were then incubated at 37 °C for 24 h under anaerobic conditions. Antimicrobial activity was determined by measuring the diameter of the growth inhibition zone. Amphotericin B was employed as the positive control.

### (d) Anthelmintic assays

Nematocidal activity was determined using the method of Lacey *et al.*<sup>28</sup> Nematode eggs were placed into wells of a microtitre plate containing test compound in 2% agar. The eggs hatched overnight, a nutrient medium was added, and the larvae were held at 27 °C for 6 days. The number of larvae that developed to the L3 stage was counted and compared to control wells.

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## Notes and references

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## Graphical contents entry

Compounds such as **5** incorporating the 3,4'-bis(piperidine) core of the marine alkaloid haliclونacyclamine A (HA, **1**) have been prepared using crossed-aldol processes. They retain most of the biological properties of the parent.

