Regioselective photo-oxidation of 1-benzyl-4,9-dihydro-3*H*-β-carbolines†

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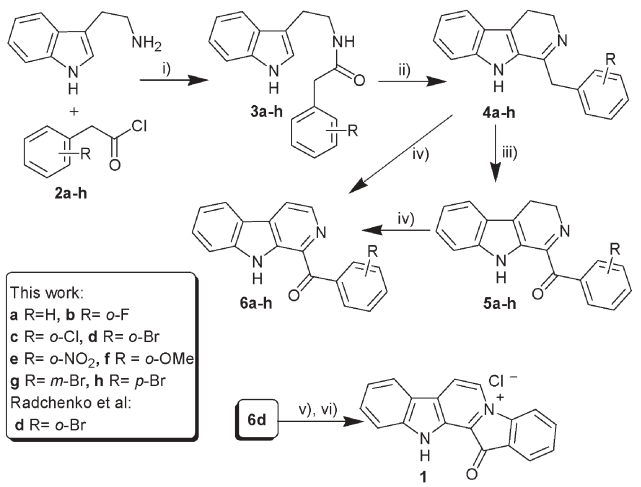
Received (in Cambridge, UK) 5th April 2006, Accepted 25th April 2006
First published as an Advance Article on the web 9th May 2006
DOI: 10.1039/b604922b

The synthesis of a series of β-carboline-based analogues of the natural product fascaplysin is presented; the compounds were produced using a novel photo-oxidation reaction of 1-benzyl-4,9-dihydro-3*H*-β-carbolines as the key step.

The natural product fascaplysin **1**, originally isolated from the Fijian sponge *Fascaplynosis* Bergquist sp,[1] inhibits the growth of several microbes including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*, and suppresses the proliferation of mouse leukaemia cells L-1210 with ED_{50} = 0.2 μm mL^{-1}. Fascaplysin **1** has also been reported to specifically inhibit CDK4 (IC_{50} = 0.55 μM) causing G_1 arrest in U2-OS and HCT-116 tumour cells, as well as normal (MRC-5) cells.[2] Fascaplysin **1** itself cannot be used as an anticancer drug because of its high toxicity, thought to be due to the fact that its flat structure can act as a DNA intercalator.[3] We have recently investigated the design, synthesis and biological evaluation of non-toxic (non-planar) analogues of fascaplysin **1** as potential specific CDK4/Cyclin D1 inhibitors.[4]

As part of this programme of research we planned to use the Radchenko approach to fascaplysin **1** (Scheme 1, R = *o*-Br)[5] for the synthesis of derivatives of the natural product.[4]



Scheme 1 Synthetic plan based on the Radchenko approach to fascaplysin **1** (R = *o*-Br). *Reagents and conditions*: i) DCM, NaOH, 0 °C to r.t., 1 h. ii) $POCl_3$, toluene, reflux, 30 min. iii) MnO_2, CH_2Cl_2, r.t., 3 h. iv. MnO_2, CH_2Cl_2, reflux, 3 h. v. 220 °C, 20 min. vi) HCl/MeOH.

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† Electronic supplementary information (ESI) available: Physical data for compounds **5a–h** and **6a–h**. See DOI: 10.1039/b604922b

In this method, the acetamide derivative **3d** is subjected to a Bischler–Napieralski reaction with $POCl_3$ in refluxing toluene; the 1-benzyl-β-carboline **4d** obtained is oxidized by MnO_2 in CH_2Cl_2 at room temperature or at reflux to afford, respectively, compounds **5d** and **6d**. Heating of compound **6d** at 220 °C for 20 minutes, followed by anion interchange with dry HCl in MeOH, leads to fascaplysin **1** with an overall 44% yield from tryptamine.

In a first attempt at reproducing the original sequence it was found that after cyclisation of the acetamide **3d**, basic work-up and column chromatography, the expected compound **4d** was isolated (58% yield) accompanied by a small amount of the oxidized product **6d** (4%).[6] The ^{1}H-NMR of compound **4d** showed two different sets of signals in a ratio of about 10 : 1; this fact was attributed to imine–enamine tautomerism (Fig. 1).

The aliphatic region in the ^{1}H-NMR spectrum of compound **4d** (Fig. 1) clearly shows the corresponding signals of the imino compound [δ_H 2.89 (t, 2H, *J* 8.5 Hz, 3-C*H*$_2$), 3.95 (t, 2H, *J* 8.5 Hz, 4-C*H*$_2$), 4.14 (s, 2H, partially exchangeable with D_2O, -C*H*$_2$-Ar)] and the peaks of the enamino tautomer [δ_H 2.97 (t, 2H, *J* 6.1 Hz, 3-C*H*$_2$), 3.41 (dt, 2H, *J* 2 and 6.1 Hz), 4.73 (br s, 1H, D_2O exch, 2-N*H*), 5.69 (s, 1H, partially exchangeable with D_2O, -C*H*-Ph)]. In the more complex aromatic region (not shown), singlet signals for the indole NHs of the imino tautomer [δ_H 8.11 (br s, D_2O exch)] and of the enamino partner [δ_H 8.29 (br s, D_2O exch)] are observed. The D_2O partial exchange of the benzylic protons (being a faster process than the auto-oxidation) confirmed the suspicion of an imine–enamine tautomerism in solution for compound **4d**. Another fact that supports this hypothesis is that the UV/Vis spectrum of the compound shows an absorption maximum near 355 nm [UV/Vis (toluene) 348 nm (log ε 1.062)], which is the typical value for the absorption of the α-aminostilbene

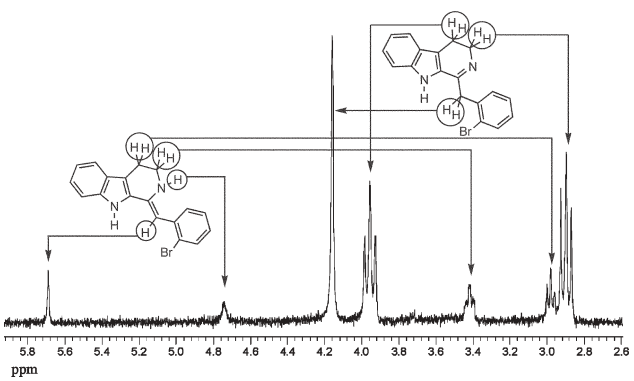


Fig. 1 Aliphatic region of the ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si) of isolated compound **4d**. Aromatic region not shown for clarity.

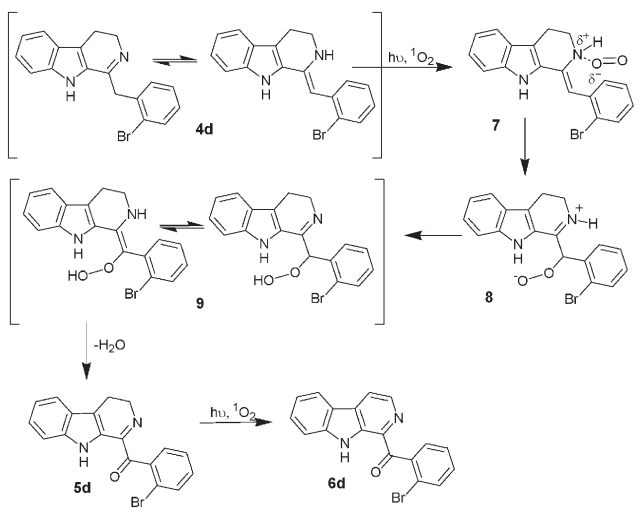
chromophore.[7] Moreover, **4d** was found to be unstable in solution; when the ^{1}H-NMR of the initial sample was recorded again two weeks later using the same sample, the spectrum showed substantial amounts of the oxidation products **5d** and **6d**.

These observations were in agreement with previous results reported by Martin *et al.*[7] who found that related 1-benzyl-3,4-dihydroisoquinolines were converted into the corresponding 1-benzoyl derivatives by means of a self-sensitized photo-oxygenation of the α-aminostilbene moiety.

The proposed mechanism of the photo-oxidation is outlined for compound **4d** (Scheme 2). A transfer of charge from the enamine-tautomer nitrogen of **4d** to singlet oxygen would form a *charge-transfer* complex **7**, which could collapse to the zwitterionic peroxide **8**, and then, by intramolecular proton transfer, to the tautomeric peroxides **9**. This intermediate could undergo dehydration to the iminoketone **5d**.[8] Finally, **5d** could undergo further photodehydrogenation to the aromatized β-carboline **6d**. As related aromatizations of dihydro-β-carbolines sensitized by methylene blue have been reported,[9] in our case the most probable explanation is that compound **5d** acts as a self-sensitizer.[10]

In an initial experiment, we tried to induce the auto-oxidation by irradiating an oxygen-bubbled solution of the purified compound **4d** in toluene with a 500 W halogen lamp (290–300 nm frequency cut off). After 16 hours at reflux, TLC analysis showed complete conversion of the initial material to a single product. Recrystallisation of the crude product yielded the compound **6d** in almost pure form (84% yield). Qualitative determination of the relative amounts of compounds **4d/5d** and **6d** during the reaction was achieved by means of ^{1}H-NMR (Fig. 2).

After 4 hours most of the starting material had been consumed. From this point the reaction comprised the transformation of the iminoketone **5d** into the fully aromatized compound **6d**. Taking these observations into account, a slight change in the reaction conditions, avoiding reflux by changing the distance between the lamp and the reaction flask (d = 5 cm → reflux, d = 25 cm → measured temperature 30 °C), allows us to obtain **5d** regioselectively in almost quantitative yield and purity after 16 hours of reaction. As expected, when **5d** was irradiated at reflux with O_2 bubbling (d = 5 cm) for 16 hours, compound **6d** was obtained after recrystallisation of the crude product with a 90% yield.



Scheme 2 Proposed mechanism for the self-sensitized photo-oxidation of **4d**.

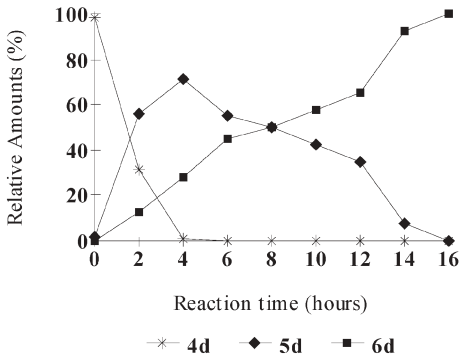
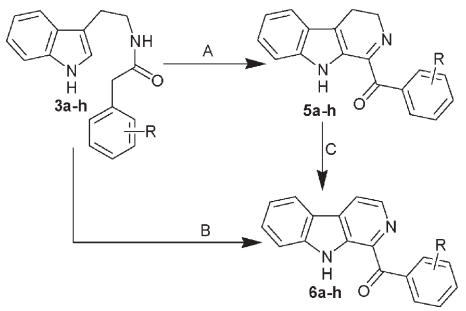


Fig. 2 Relative amounts of compounds **4d/5d** and **6d** during the irradiation of **4d** in toluene at reflux. The relative ratios were directly measured from the ^{1}H-NMR spectra; the peaks chosen for this purpose were δ_H = 4.14 ppm (-C*H*$_2$-Ar, **4d**), δ_H = 9.48 ppm (indolyl N*H*, **5d**) and δ_H = 10.43 ppm (indolyl N*H*, **6d**).

The synthetic scope of this mild, green and selective oxidation for the synthesis of compounds **5a–h** and **6a–h** was investigated; the results are summarized in Table 1.

Table 1 Results for the conversion of **3a–h** into **5a–h/6a–h** and **5a–h** into **6a–h**[a]



Entry	R	Method	[b]Time/h	[c]Product	[d]Yield (%)
1	H	A	16	**5a**	50
2	*o*-F	A	16	**5b**	57
3	*o*-Cl	A	16	**5c**	59
4	*o*-Br	A	16	**5d**	48
5	*o*-NO_2	A	16	—	decomposition
6	*o*-OMe	A	16	**5f/6f**: 1/0.25	31/10
7	*m*-Br	A	16	**5g**	46
8	*p*-Br	A	16	**5h**	44
9	*o*-F	B	16	**6b**	11
10	*o*-Cl	B	32	**6c**	29
11	*o*-Br	B	16	**6d**	49
12	*o*-OMe	B	16	**6f**	55
13	*p*-Br	B	72	**6h**	44
14	H	C	48	**6a**	99
15	*o*-F	C	16	**6b**	62
16	*o*-Cl	C	32	**6c**	99
17	*o*-Br	C	16	**6d**	99
18	*m*-Br	C	48	**6g**	99

[a] *Reagents and conditions* (see footnote[11] for experimental details): A: i) $POCl_3$, toluene, N_2, reflux. ii) basic work-up. iii) *hν*, O_2, toluene, 30 °C; B: i) $POCl_3$, toluene, N_2, reflux. ii) basic work-up. iii) *hν*, O_2, toluene, reflux; C: i) *hν*, O_2, toluene, reflux. [b] Reactions monitored by ^{1}H-NMR. [c] Isolated compounds after purification of crude reaction mixture. [d] Yields of the purified products from the tryptamine derivatives **3** (entries 1–13) or ketoimines **5** (entries 14–18).

To circumvent the intrinsic instability of compounds **4a–h**, purification after cyclisation of the corresponding acetamide derivatives **3a–h** was avoided, so the crude reaction product after basic work-up of the Bischler–Napieralski reaction was irradiated under the conditions explained in Table 1.

Using the method A (entries 1–8), regioselectivity in the oxidation was achieved in most of the cases, yielding the dihydro-β-carbolines **5a–h** with satisfactory yields from the corresponding tryptamine derivatives **3a–h**. When d = 5 cm [method B, entries 9–13] reflux of the toluene solution occurred and, consequently, the fully aromatic β-carbolines **6a–h** were isolated with acceptable yields from **3a–h**. On other occasions the compounds **6a–h** were obtained in good yields by irradiation (d = 5 cm) of the isolated ketoimines **5a–h** [method C, entries 14–18].

Finally, a one-pot sequence for the synthesis of the target compounds was also explored. Tryptamine was reacted with 2-bromophenylacetic chloride **2d** (1.1 eq.) in toluene at reflux for 15 minutes; $POCl_3$ (2.5 eq.) was then added to the mixture. Once TLC analysis of a worked-up aliquot showed complete reaction, the crude mixture was irradiated with the 500 W halogen lamp and oxygen bubbled throughout the solution. After 24 hours at reflux, the only isolated product was **4d** in a 48% yield from tryptamine. One probable explanation for the inhibition of the oxidation is that the product of the Bischler–Napieralski reaction before the basic work-up should be the hydrochloride of compound **4d**. In such a situation the imine–enamine tautomerism is inhibited, so the charge transfer between the enamine NH and singlet oxygen required for the oxidation does not occur.

In conclusion, we have investigated the spontaneous photo-oxidation of the 1-(2-bromobenzyl)-4,9-dihydro-3*H*-β-carboline; this has led to the development of a mild, regioselective and practical protocol for the preparation of dihydro-β-carbolines of type **5** and β-carbolines of type **6** from tryptamine derivatives **4** by a sequential cyclisation-induced photo-oxidation of the non-isolated 1-benzyl-4,9-dihydro-3*H*-β-carboline derivatives.

A separate investigation into the biological activity of compounds **5/6**, and their optimization as potential inhibitors of CDK4/Cyclin D1 is currently underway.

This work was supported by Cancer Research UK. M. D. G. thanks the Xunta de Galicia for financial support.

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6 This observation is not new, since related compounds of type **5/6** have been isolated after basic work-up of the Bischler–Napieralski reaction and it has been linked to the spontaneous oxidation of an initially formed **4**-type compound; K. M. Biswas and A. H. Jackson, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1981.

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8 We propose that the photo-oxidation proceeds *via* a charge-transfer complex. The precedent for this comes from the work of Martins *et al.*[7] who proposed a similar mechanism in a related isoquinoline case. The alternative concerted ene mechanism was discounted on the basis of structure–activity studies.

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11 Examples of general procedures A, B and C (Table 1): **Method A: synthesis of 1-(2-bromobenzoyl)-3,4-dihydro-β-carboline 5d from 2-(2-bromophenyl)-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide 3d**. The acetamide derivative **3d** (1 mmol, 357 mg) was heated in toluene until complete dissolution was achieved. $POCl_3$ (10 mmol, 0.93 mL) was added and the resulting mixture refluxed for 1 hour until TLC analysis showed completion. Afterwards, toluene was removed and the residue dissolved in 20 mL of a 2 : 1 mixture of CH_2Cl_2/$NaHCO_3$. The biphasic mixture was then cooled and basified with aqueous NH_3 to pH 9. The organic phase was separated and the aqueous layer extracted with CH_2Cl_2 (2 × 25 mL). Then, the combined organic phases were dried over sodium sulfate and the solvent evaporated. The crude product **4d** obtained was suspended in toluene with vigorous stirring; oxygen was bubbled through the solution and the mixture was irradiated with a 500 W halogen lamp located at 25 cm from the reaction flask (measured temperature of the suspension *ca.* 30° C). The reaction was monitored by ^{1}H-NMR until completion. Then, the toluene was evaporated under reduced pressure and the crude product obtained (410 mg) filtrated over a short pad of SiO_2 (2 g of SiO_2) eluting with CH_2Cl_2 to give **5d** as an orange solid (170 mg, 48%); mp 136–137 °C (from EtOH); Found: C, 61.14; H, 3.72; N, 7.88%; $C_{18}H_{13}BrN_2O$ requires: C, 61.21; H, 3.71; N, 7.93%; ν_{max}/cm^{-1} 3453, 1660, 1585, 1539, 1437, 1295, 1222, 1143, 732; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.0 (2H, t, *J* 8.9 Hz), 4.13 (2H, t, *J* 8.9 Hz), 7.14–7.19 (1H, m), 7.30–7.53 (4H, m), 7.61 (1H, d, *J* 1.2 Hz), 7.61 (1H, d, *J* 1.2 Hz), 7.64 (1H, d, *J* 1.2 Hz) and 9.48 (1H, br s, D_2O exch, NH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ 18.8 (CH_2), 49.7 (CH_2), 112.3 (CH), 118.2 (Cq), 120.0 (CH), 120.3 (Cq), 120.4 (CH), 124.7 (Cq), 125.2 (CH), 126.1 (Cq), 127.0 (CH), 129.8 (CH), 131.9 (CH), 133.1 (CH), 137.1 (Cq), 139.1 (Cq), 155.5 (Cq) and 196.5 (CO) ppm; *m*/*z* (ES$^+$) 353 (MH$^+$); *m*/*z* (FAB$^+$) 353 (MH$^+$) (found: MH$^+$, 353.02897; $C_{18}H_{14}BrN_2O$ requires 353). **Method B: synthesis of 1-(2-bromobenzoyl)-β-carboline 6d from 2-(2-bromophenyl)-*N*-[2-(1*H*-indol-3-yl)ethyl]acetamide 3d**. The crude product **4d** obtained from the acetamide derivative **3d** (1 mmol, 357 mg) as described in method A was irradiated following the protocol described in method A with the 500 W halogen lamp located at 5 cm from the reaction flask so reflux was achieved. The mixture was refluxed until completion (monitored by ^{1}H-NMR). The crude product (430 mg) obtained after evaporation of the toluene was purified as in method A yielding **6d** (172 mg, 49%) as an orange solid; mp 215–216 °C (from EtOH/H_2O); Found: C, 61.41; H, 3.09; N 7.92%; $C_{18}H_{11}N_2OBr$ requires: C, 61.56; H, 3.16; N, 7.98%; ν_{max}/cm^{-1} 3447, 1654, 1581, 1186, 1174, 950, 792, 775, 746; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.35–7.42 (2H, m), 7.47 (1H, dt, *J* 1.3 and 7.3 Hz), 7.56 (1H, dd, *J* 1.7 and 7.3 Hz), 7.63 (1H, d, *J* 0.9 Hz), 7.64 (1H, q, *J* 0.9 Hz), 7.69 (1H, dd, *J* 1.0 and 8.0 Hz), 8.16–8.20 (2H, m), 8.56 (1H, d, *J* 5 Hz) and 10.43 (1H, br s, D_2O exch, NH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ 112.1 (CH), 119.2 (CH), 120.1 (Cq), 120.7 (Cq), 121.0 (CH), 121.9 (CH), 126.9 (CH), 129.5 (CH), 129.8 (CH), 131.2 (CH), 131.9 (Cq), 133.1 (CH), 135.2 (Cq), 136.9 (Cq), 138.8 (CH), 140.4 (Cq), 141.2 (Cq) and 198.2 (CO) ppm; *m*/*z* (ES$^+$) 351 (MH$^+$); *m*/*z* (FAB$^+$) 351 (MH$^+$) (found: MH$^+$, 351.01321; $C_{18}H_{12}BrN_2O$ requires 351). **Method C: synthesis of 1-(2-bromobenzoyl)-β-carboline 6d from 1-(2-bromobenzoyl)-3,4-dihydro-β-carboline 5d**. Compound **5d** (176 mg, 0.5 mmol) obtained as described in method A was irradiated with the 500 W halogen lamp as in method B. The reaction was monitored by ^{1}H-NMR until completion and the resulting crude product obtained was purified as described to yield **6d** (350 mg, 99%), which displayed spectroscopic features identical to those described above.

