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A biomimetic synthesis of calothrixin B

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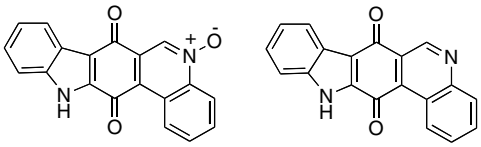
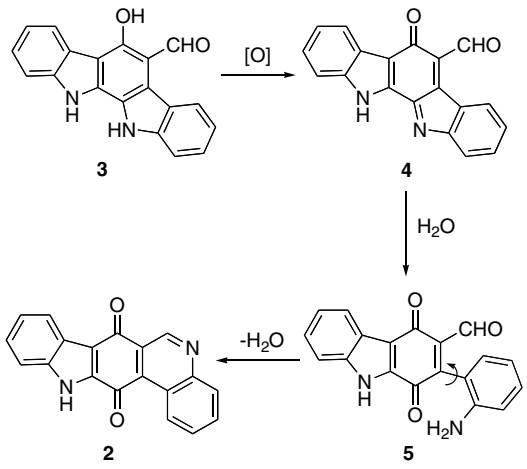
Abstract—Oxidation of the indolo[2,3-*a*]carbazole **15**, readily obtained in six steps from indigo, followed by deprotection results in formation of the indolo[3,2-*j*]phenanthridine quinone alkaloid calothrixin B **2**, demonstrating the viability of the proposed biosynthetic route to this unique ring system.

Calothrixin A **1** and its *N*-deoxy derivative calothrixin B **2** are heterocyclic quinones isolated by Rickards et al. in 1999 from a *Calothrix* cyanobacterium.[1] These alkaloids exhibit remarkable biological activity, most notably their growth inhibitory effects at nanomolar concentrations on a chloroquine resistant strain of the malarial parasite *Plasmodium falciparum*, as well as activity against human HeLa cancer cells, and inhibition of RNA polymerase activity.[1,2]



1 **2**

The pentacyclic indolo[3,2-*j*]phenanthridine ring system of the calothrixins is unique amongst natural products and was proposed to arise in Nature from the more common indolo[2,3-*a*]carbazole framework.[1] This fascinating proposal by Rickards et al. for the biogenesis of the calothrixins is outlined in Scheme 1, and involves the oxidation of the putative indolocarbazole intermediate **3**. The resulting quinoneimine **4** could undergo hydrolysis to the anilino-quinone **5**, which after rotation around the biaryl bond and condensation of the amino group with the aldehyde would give calothrixin B (Scheme 1).



Scheme 1. Proposed biogenesis of the indolo[3,2-*j*]phenanthridine ring by the oxidation of an indolo[2,3-*a*]carbazole.

Due to their potent biological activity and unique ring system, these alkaloids have attracted much attention from synthetic chemists. The first total synthesis was reported by Kelly and co-workers, and involved coupling of indole and isoquinoline fragments.[3] Subsequently, further syntheses from the groups of Chai, Guingant, Hibino and Bennasar have been reported.[4–7] In view of our interest in heterocyclic quinones,[8] we were intrigued by the Rickards proposal for the biosynthesis of

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the calothrixins skeleton, and initiated a programme designed to test that hypothesis, beginning with the synthesis of indolo[2,3-*a*]carbazole **3** from commercially available indigo.[9] The very recent report by Hibino and co-workers on a similar biogenetically patterned synthesis of calothrixin B,[10] prompts us to disclose our own investigations in this field.

Our route to the key indolo[2,3-*a*]carbazole carboxaldehyde **3** is based on the synthesis of 5-cyano-6-methoxy-12-methylindolo[2,3-*a*]carbazole, a cytotoxic natural product isolated from the blue–green alga *Nostoc sphaericum*,[11] developed by Somei and co-workers.[12] Thus a mixture of indigo and tin powder was heated under carefully controlled conditions (64–66 °C) in a solution of acetic anhydride in acetic acid to give mono-acetoxylated bis-indole **6**, which was then acylated with dichloroacetyl chloride. Treatment of the resulting bis-indole **7** with aqueous ammonia effected ring closure and gave *cis*-chlorohydrin **8**, whose structure and stereochemistry was confirmed by X-ray crystallographic analysis (Fig. 1).[13,14] The reduction of chlorohydrin **8** gave the known 5-hydroxyindolo[2,3-*a*]carbazole **9**, and subsequent Vilsmeier formylation delivered the desired indolocarbazole-5-carboxaldehyde **3** (Scheme 2).[15]

In an attempt to affect the proposed biomimetic transformation, indolocarbazole **3** was subjected to a plethora of oxidative conditions [including reagents such as chloranil, DDQ, $NaIO_4$, CAN, $Pb(OAc)_4$, MnO_2, AgO, H_2O_2, Fremy's salt, and hypervalent iodine reagents], all without success. The universal failure of these reagents to produce the calothrixin framework led us to consider that the second *NH* on the indolo[2,3-*a*]-carbazole skeleton may be adversely affecting the oxidation. To test whether a mono-N-protected indolocarbazole would undergo the desired biomimetic oxidation-rearrangement, compound **12** was synthesised. Thus, chlorohydrin **8** was methylated by the action of dimethyl sulfate to give **10**. Reduction and subsequent Vilsmeier formylation delivered the mono-protected indolocarbazole **12**, whose structure was confirmed by X-ray crystallography (Fig. 2).[13,16] Disappointingly, subjecting this compound to a similar battery of oxidative reagents did not result in the isolation of the desired *N*-methyl-calothrixin B **14** (Scheme 3).

The failure of a range of oxidation methods prompted an electrochemical study of the indolocarbazole ring system. Cyclic voltammetry measurements indicated that an irreversible two-electron oxidation could be achieved

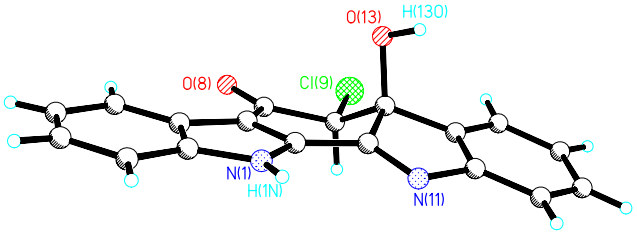
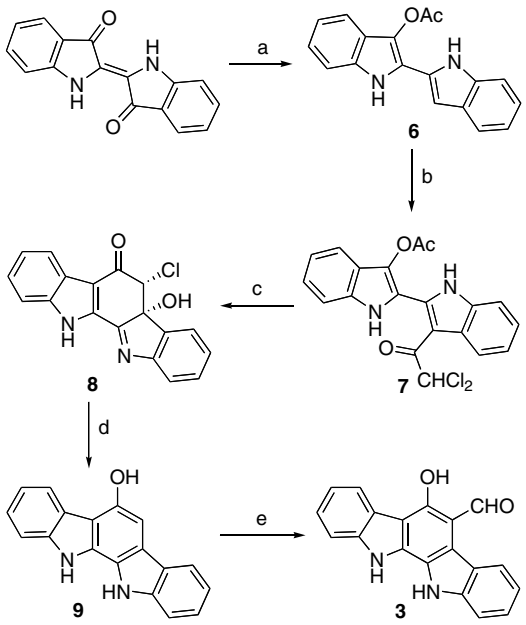


Figure 1. X-ray structure of chlorohydrin **8**.



Scheme 2. Reagents and conditions: (a) Sn, Ac_2O, AcOH, 64–66 °C (85%); (b) $Cl_2CHCOCl$, EtOAc, reflux (79%); (c) aq NH_3, DMF, MeOH (75%); (d) Zn, NH_4Cl, THF, MeOH (55%) and (e) $POCl_3$, DMF (82%).

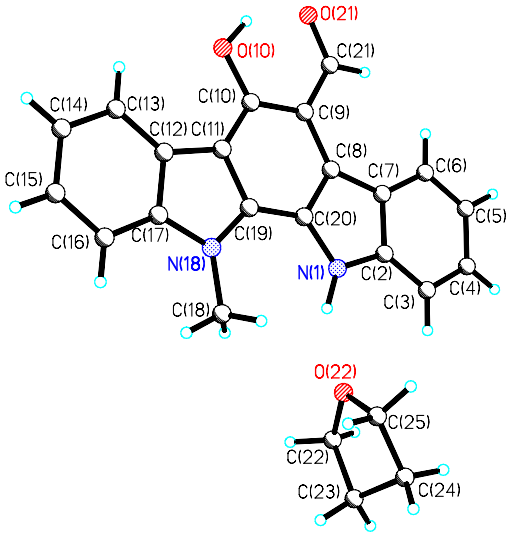
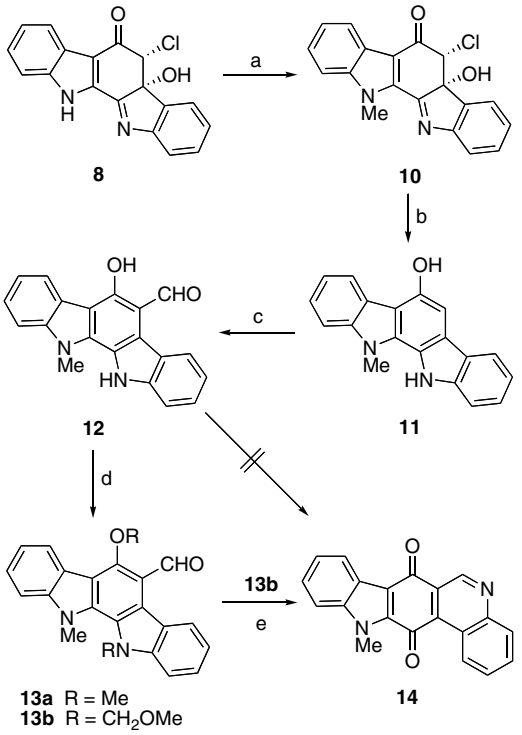


Figure 2. X-ray structure of indolecarbazole-5-carboxaldehyde **12** (crystallised with one molecule of THF).

for compound **12**, although the oxidation potential was relatively high. Encouraged by these data, we next investigated the oxidation of further derivatives of indolocarbazole **12**. Although the trimethyl compound **13a** did not undergo oxidation when treated with cerium(IV) ammonium nitrate (CAN), the use of an alternative alkyl group, the methoxymethyl (MOM) group, proved successful. Thus, bis-MOM compound **13b** gratifyingly underwent oxidation and in situ rearrangement to give *N*-methyl-calothrixin B **14** in a 52% yield,[17] thereby demonstrating that the biosynthetic transformation

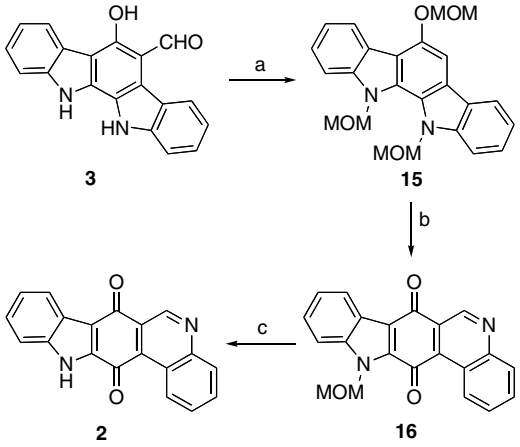


Scheme 3. Reagents and conditions: (a) Me_2SO_4, K_2CO_3, acetone (100%); (b) Zn, NH_4Cl, THF, MeOH (61%); (c) $POCl_3$, DMF (82%); (d) for **13a**; LDA, THF, 0 °C, MeI (52%); for **13b**; LHMDS, THF, 0 °C, $MeOCH_2Cl$ (60%) and (e) CAN, aq MeCN (52%).

proposed by Rickards could be reproduced under laboratory conditions.

With this new found knowledge about the need to alkylate the phenolic *OH* and indolic *NH* groups in the putative biosynthetic precursor indolocarbazole-5-carboxaldehyde **3** for a successful oxidative transformation of the ring system into the indolo[3,2-*j*]phenanthridine-quinone, the synthesis of calothrixin B **2** could be accomplished. As in the first synthesis of calothrixin B by Kelly et al.,[3] we elected to use the readily removable methoxymethyl group as the indole NH protecting group. Thus, indolocarbazole **3** was treated with excess chloromethyl methyl ether to give the tris-MOM-protected compound **15**, an intermediate also employed in Hibino's approach.[10] Oxidation was affected by the action of cerium(IV) ammonium nitrate, and subsequent in situ hydrolysis, bond rotation and condensation delivered *N*-MOM-calothrixin B **16** in a moderate yield.[18] Finally, deprotection under acidic conditions gave calothrixin B **2** (Scheme 4), whose NMR spectroscopic properties were identical to those of an authentic sample of the natural product.

In conclusion, we have investigated the proposed biomimetic oxidative transformation of the indolo[2,3-*a*]carbazole framework into the indolo[3,2-*j*]phenanthridine quinone ring system. Whilst the unprotected compounds failed to undergo the desired reaction cascade, a judicious use of protecting groups facilitated oxidation and subsequent rearrangement. This methodology was



Scheme 4. Reagents and conditions: (a) $MeOCH_2Cl$, NaH, DMF (44%); (b) CAN, aq MeCN (31%) and (c) concd HCl, THF, 55 °C (100%).

then utilised to complete a biomimetic synthesis of calothrixin B.

Acknowledgements

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14. (4*aR**,5*R**)-5-Chloro-4*a*-hydroxy-6-oxo-4a,5,6,11-tetrahydroindolo[2,3-*a*]carbazole **8**; yellow solid, mp 229–231 °C (from ethyl acetate/hexanes, 1:1); δ_H (400 MHz; DMSO-d_6) 13.11 (1H, br s, NH), 8.14 (1H, d, *J* 8.1, ArH), 7.80 (1H, d, *J* 7.2, ArH), 7.71 (1H, d, *J* 7.2, ArH), 7.59 (1H, d, *J* 8.1, ArH), 7.52 (1H, t, *J* 7.6, ArH), 7.42 (1H, t, *J* 7.2, ArH), 7.36 (1H, t, *J* 7.4, ArH), 7.33 (1H, t, *J* 7.4, ArH), 6.78 (1H, s, OH), 5.38 (1H, s, CH); δ_C (75 MHz; DMSO-d_6) 185.4 (C), 169.2 (C), 155.2 (C), 139.7 (C), 139.0 (C), 137.1 (C), 130.8 (CH), 127.0 (CH), 126.4 (CH), 125.7 (CH), 124.6 (C), 123.5 (CH), 122.1 (CH), 121.5 (CH), 115.9 (C), 113.4 (CH), 87.1 (C), 70.3 (CH).
15. 6-Hydroxyindolo[2,3-*a*]carbazole-5-carbaldehyde **3**; orange solid, mp > 275 °C (from THF/light petroleum); δ_H (400 MHz; DMSO-d_6) 13.86 (1H, s, OH), 12.23 (1H, s, NH), 11.63 (1H, s, NH), 10.92 (1H, s, CHO), 8.30 (1H, d, *J* 8.1, ArH), 8.24 (1H, d, *J* 8.1, ArH), 7.71–7.68 (2H, m, ArH), 7.45–7.38 (2H, m, ArH), 7.30 (1H, t, *J* 7.3, ArH), 7.18 (1H, t, *J* 7.3, ArH); δ_C (100 MHz; DMSO-d_6) 192.9 (CH), 158.7 (C), 140.0 (C), 139.3 (C), 133.4 (C), 125.53 (CH), 125.51 (CH), 123.6 (C), 123.5 (CH), 122.32 (C), 122.27 (CH), 121.5 (CH), 120.6 (C), 120.2 (CH), 118.3 (C), 113.0 (CH), 112.6 (CH), 108.7 (C), 108.4 (C).
16. 6-Hydroxy-11-methylindolo[2,3-*a*]carbazole-5-carbaldehyde **12**; orange solid, mp > 275 °C (from THF/light petroleum); δ_H (400 MHz; DMSO-d_6) 13.83 (1H, s, OH), 11.67 (1H, s, NH), 10.93 (1H, s, CHO), 8.31–8.27 (2H, m, ArH), 7.75–7.69 (2H, m, ArH), 7.51–7.47 (1H, m, ArH), 7.44–7.40 (1H, t, *J* 7.2, ArH), 7.31 (1H, t *J* 7.6, ArH), 7.17 (1H, t, *J* 8.0, ArH), 4.34 (3H, s, Me); δ_C (75 MHz; DMSO-d_6) 192.6 (CH), 158.5 (C), 140.8 (C), 140.5 (C), 140.5 (C), 134.4 (C), 125.5 (CH), 125.4 (CH), 123.4 (CH), 123.0 (C), 122.3 (CH), 121.7 (CH), 121.6 (CH), 120.2 (C), 120.1 (C), 119.3 (CH), 112.9 (CH), 110.4 (C), 108.3 (C), 34.8 (Me).
17. 12-Methylindolo[3,2-*j*]phenanthridine-7,13-dione (*N*-methyl-calothrixin B) **14**; orange solid, mp > 230 °C; δ_H (500 MHz; $CDCl_3$) 9.81 (1H, s, CHN), 9.61 (1H, d, *J* 9.6 Hz, ArH), 8.45 (1H, d, *J* 7.9, ArH), 8.21 (1H, d, *J* 7.1, ArH), 7.86 (1H, td, *J* 6.8, 1.3, ArH), 7.79 (1H, td, *J* 7.9, 1.3, ArH), 7.55–7.41 (3H, m, ArH), 4.31 (3H, s, NMe); δ_C (125 MHz; $CDCl_3$) 182.3 (C), 180.8 (C), 152.8 (C), 147.9 (C), 138.6 (C), 138.3 (C), 131.3 (CH), 130.3 (CH), 130.1 (CH), 127.76 (C), 127.70 (CH), 127.5 (CH), 125.3 (C), 125.0 (CH), 124.3 (C), 123.83 (CH), 123.80 (CH), 110.9 (CH), 109.9 (C), 32.3 (Me).
18. 12-Methoxymethylindolo[3,2-*j*]phenanthridine-7,13-dione (*N*-methoxymethyl-calothrixin B) **16**; orange solid, mp > 230 °C (lit., [Ref. 3] mp 234–235 °C); δ_H (500 MHz; $CDCl_3$) 9.80 (1H, s, CHN), 9.61 (1H, d, *J* 8.5 Hz, ArH), 8.46 (1H, d, *J* 8.0, ArH), 8.22 (1H, d, *J* 8.0, ArH), 7.86 (1H, td, *J* 8.0, 1.3, ArH), 7.78 (1H, td, *J* 8.5, 1.3, ArH), 7.67 (1H, d, *J* 8.0, ArH), 7.56 (1H, t, *J* 8.0, ArH), 7.53 (1H, t, *J* 8.0, ArH), 6.18 (2H, s, NCH_2OMe), 3.39 (3H, s, $NCH_2O\mathit{Me}$); δ_C (100 MHz; $CDCl_3$) 182.1 (C), 181.4 (C), 152.3 (C), 147.8 (CH), 140.2 (C), 135.4 (C), 133.3 (C), 131.5 (CH), 130.4 (CH), 130.2 (CH), 128.3 (CH), 127.6 (CH), 125.3 (CH), 124.3 (C), 123.9 (CH), 123.3 (C), 123.2 (C), 118.6 (C), 111.9 (CH), 75.4 (CH_2), 56.6 (Me).