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Cascade reactions of indigo with oxiranes and aziridines: efficient access to dihydropyrazinodiindoles and spiro-oxazocinodiindoles

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The base-initiated alkylation of the abundant natural dye indigo **1** with ring-strained electrophiles results in the unprecedented, one-pot synthesis of functionalised dihydropyrazino[1,2-*a*:4,3-*a*']diindoles, dihydroepoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindoles, and dihydrodiazepino[1,2-*a*:4,3-*a*']diindoles, resulting from intramolecular ring opening-expansion cyclisation processes of their parent oxiranes and aziridines. Regiochemical and stereochemical aspects of the reactions are reported together with integrated mechanistic proposals. This new indigo cascade chemistry should have broad applicability in the synthesis of chemical architectures, not readily-accessible by other means. The three-step synthesis of the useful synthetic precursor (*R*)-2-(chloromethyl)-1-tosylaziridine **14** is also described. Initial biological activity investigations into these new 2,2'-dindolyl-based heterocyclic derivatives revealed potent, selective antiplasmodial activity *in vitro* for several isolated structures, with IC_{50} values as low as 76.6 nM for (±)-**8**, while demonstrating low human cell toxicity.

Introduction

There has been a resurgence in the fields of both discovery- and diversity-oriented synthesis as tools for the realisation of new molecular architectures, particularly through multi-bond-forming cascade reactions.[1-4] Our fortuitous discovery of the complex cascade chemistry of indigo (**1**) and its family has allowed access to a range of new 2,2'-diindole-based systems, after initial base-induced *N*-alkylation of indigo in procedurally-simple, one-pot reactions (Scheme 1).[5-8]

The synthesis of 2,2'-biindole derivatives is of particular importance, as they constitute a broad family of molecules with diverse bioactivity profiles, including as kinase inhibitors, antimicrobials and antiparasitic agents.[9-14] Conceptually, indigo would represent an ideal starting point for the synthesis of such molecules as it is produced annually on kilo-tonne scales for the dyeing of denim and other textiles, however despite its popularity and colourful history, there is a surprising dearth of understanding about the accessible chemistry of indigo.

We have previously reported the *N*-alkylation of indigo with allylic and propargylic halides, which initiate a complex domino process where the electron-rich C=C or C≡C bond reacts with the indigo nucleus, where the nature of the terminal π-bond plays a defining role in the pathway direction as the cascade proceeds. Spirocyclic products predominate with the use of allylic bromides, resulting from nucleophilic attack of the allyl C=C bond on the indigo C2 position, while the rigidity of the propargyl pendant served to suppress this pathway, instead leading to products resulting from either further N-alkylation and subsequent cyclisation, or strain-driven ring-expansion (Scheme 1). Encouraged by the promising antiplasmodial activity of several derived species from these initial investigations,[6] we wished to further explore this pathway divergence, with a view to accessing spirocyclic scaffolds incorporating new heteroatoms to assess their biological relevance. Thus we investigated the reactions of indigo in the presence of base with alkyl halides incorporating a terminal oxirane or aziridine group. We hypothesised that ring-opening of these strained, electrophilic systems by the indigo core could be utilised as a trigger for further reactions with the newly-exposed nucleophilic heteroatom, promoting the cascade process. Furthermore, the prevalence and availability of chiral oxiranes and aziridines would allow access to these geometries in a stereospecific fashion. Therefore, as a direct three-carbon analogue to previously-explored propargylic and allylic substrates, we explored the reactivity of indigo with (halomethyl)oxiranes and aziridines, as well as selected four-carbon homologues in their dimeric bioxirane and biaziridine counterparts, in order to probe their utility toward the

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Electronic Supplementary Information (ESI) available: [Full experimental procedures and ^{1}H and ^{13}C NMR spectra for all compounds and HPLC traces and crystallographic data for .]. See DOI: 10.1039/x0xx00000x

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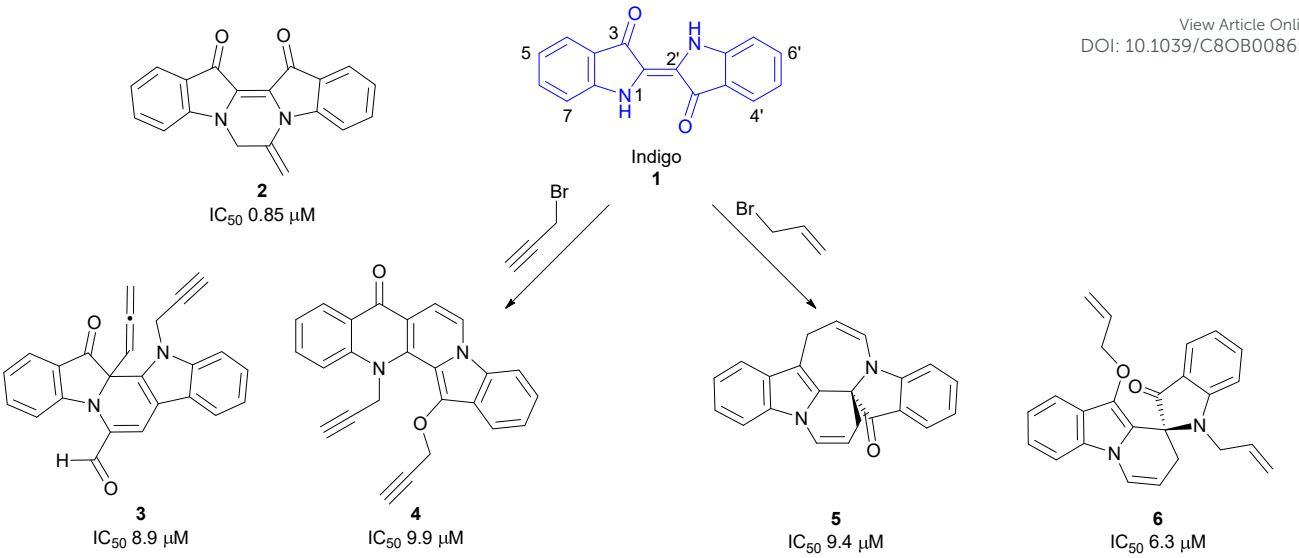


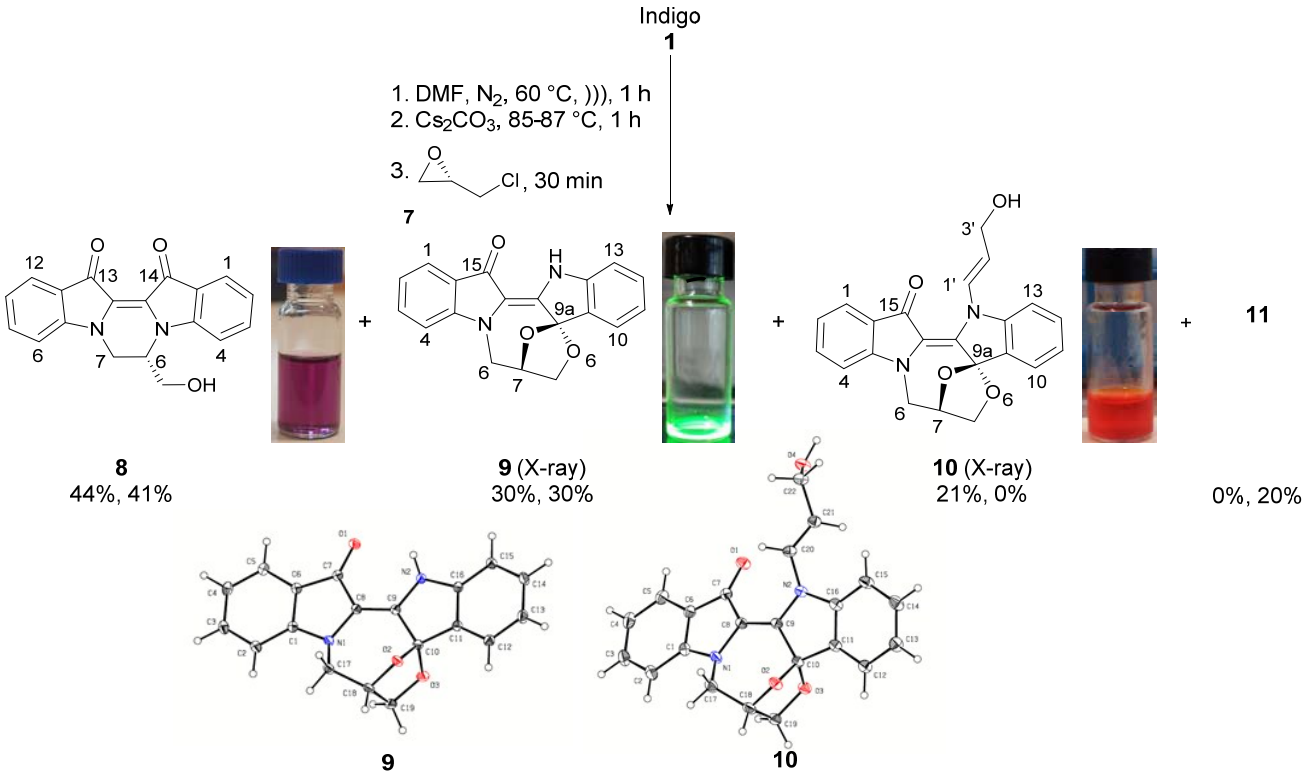
Scheme 1. Selected examples of molecules isolated from cascade reactions of indigo with allylic and propargylic bromides, with associated IC_{50} data *in vitro* against *P. falciparum* (K1 strain).

construction of new poly-heterocyclic frameworks, and to probe the antiplasmodial potential of these compounds.

Results and Discussion

Reactions of indigo with 2-(halomethyl)oxiranes and 2,2'-bioxirane

Our previous experience with the cascade reactions of indigo demonstrated that small variations in reaction conditions can lead to major changes in the outcome of the reaction.[7] Therefore, under optimised conditions, a warmed, sonicated suspension of indigo (**1**) in DMF was reacted with pre-dried and ground cesium carbonate (3.7 eq.), and the mixture heated for one hour in an oil bath preheated to strictly 85–87 ˚C under an inert nitrogen atmosphere. The nitrogen flow was cut, then (*S*)-epichlorohydrin (**7**, optimised to 5.0 eq.) injected, and the mixture stirred vigorously at 85–87 ˚C for 30 minutes, resulting in the isolation of three compounds (Table 1) – the *N*,*N*'-cyclised



Scheme 2. Base-mediated cascade reaction of indigo producing heterocycles **8**-**10**, with ORTEP depiction of compounds **9**, and **10**. Adjacent to compounds **8**-**10** is pictured a solution of each in dichloromethane, highlighting the colour. Yields reflect Table 1, Entries 1 and 6 respectively. The synthesis of compound **10** was variable and was isolated with a small amount of grease contaminant present, as analysed by NMR, which could not be removed despite repeated attempts. For the structure of **11**, please see Scheme 3. Note: The X-ray structure atom numbers are different from the systematic atom numbers.

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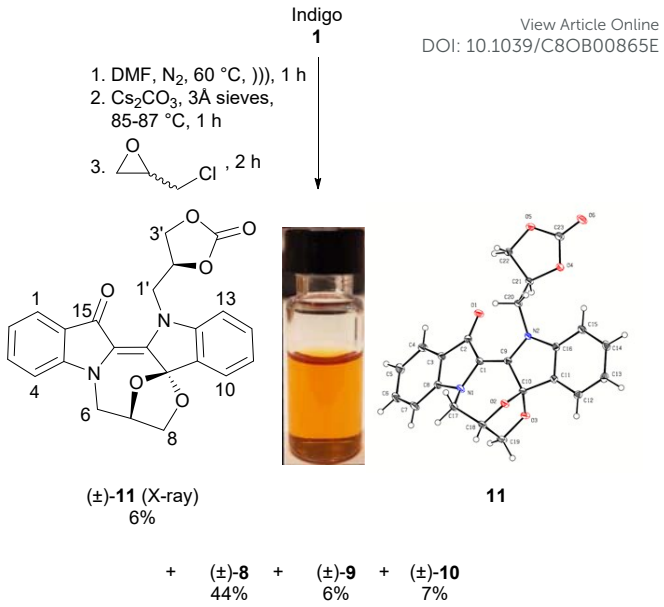
8, and *N*,*O*'-spirocyclised products **9** and **10** – in a combined 95% yield (Scheme 2).

The major product was the dark blue *N*,*N*'-cyclised dihydropyrazinodiindole **8**, which was isolated in 44% yield by recrystallisation from the crude reaction mixture. Analysis of the HRESI mass spectrum indicated a peak at *m/z* 341.0888, assigned as $[C_{19}H_{14}N_2O_3Na]^+$, revealing the addition of a single glycidyl subunit to the indigo core. Analysis of the ^{1}H NMR spectrum showed a broad singlet resonance at 5.28 ppm which exchanged with D_2O and showed no direct C-H attachment through gHSQC analysis, and was assigned as a hydroxyl group. Analysis of the ^{13}C-APT spectrum indicated the retention of both carbonyls of the parent scaffold, assigned to resonances at 179.9 and 179.7 ppm. The gHMBC spectrum revealed strong, three-bond correlations between methine H6 and both C4a, and C13b; and methylene H7 with both C8a, and C13a, respectively, and the alkyl chain was therefore determined to bridge both indigo nitrogen atoms.

The spiroketal compound **9** was isolated as orange plate crystals in 30% yield, and showed brilliant yellow-green fluorescence in CH_2Cl_2 solution, both in ambient light, and under UV light (365 nm). Analysis of the HRESI mass spectrum revealed the addition of a single glycidyl subunit by a peak at *m/z* 341.0900, assigned as the $[M+Na]^+$ molecular ion. The rigid, spirocyclic alkyl tether was assigned by analysis of the gHMBC spectrum, which showed a strong three-bond correlation between three proton resonances at 4.26, 4.37, and 5.08-5.16 ppm (assigned as H8a, H8b and H7, respectively) to a quaternary carbon at 110.9 ppm, assigned as C9a. ^{13}C-APT spectral analysis revealed a single carbonyl resonance at 188.1 ppm, indicating that one carbonyl remained intact. There was no evidence of peak doubling in the ^{13}C-spectrum, suggesting the compound to be free of multiple diastereomers, and x-ray crystallography confirmed the structure of **9**, as well as the relative stereochemical configuration at the C7 and C9a carbon atoms.

Compound **10** was isolated as orange plate crystals in 21% yield. Analysis of the gHSQC spectrum indicated three methylene carbons at 52.2, 61.9, and 67.6 ppm, which were assigned as C6, C3', and C8, respectively by analysis of the zTOCSY spectrum, which showed long-range coupling between ^{1}H resonances at 3.74 and 4.27 ppm, assigned as H6a and H8, respectively. Further analysis of the ^{1}H-spectrum revealed the presence of a pair of spin-coupled resonances at 6.07 and 6.87 ppm, assigned to H2' and H1' respectively in the *trans*-enamine moiety, which coupled with the C3'-methylene by COSY and zTOCSY experiments. The multiplet ranging from 5.01–5.05 ppm was assigned as H7 by gCOSY analysis, which showed correlations to both H6 and H8, allowing complete assignment of the spirocyclic ring, as confirmed by x-ray crystallography. Despite its isolation from numerous reactions during the optimisation process, its synthesis was variable.

Dihydropyrazinodiindole **8** was the major product for all reactions of indigo with epichlorohydrin derivatives, and interestingly, the substitution of the chloride leaving group for the more reactive bromide (Table 1, entry 8) or tosylate (entry 9) groups resulted in the sole isolation of **8** in high yield (84% and 79% respectively), while in contrast, the addition of a KI



Scheme 3. Reaction of indigo with (±)-epichlorohydrin (**7**) to give spirocycle (±)-**11**, and the ORTEP drawing of racemic **11**. Yields reflect Entry 3, Table1. Adjacent to the structure is a picture of a solution of **11** in dichloromethane, showing its colour in ambient light.

catalyst (entry 5) resulted in mainly polymeric baseline materials. Product distribution correlated with the reaction time when using (halomethyl)oxiranes; longer reaction times typically led to diminished overall recovery and increased isolation of polymeric baseline materials. The *N*,*O*'-spirocycle (**11**), not seen in the original reaction (Scheme 2, Table 1, Entry 1), was isolated as orange plate crystals in 6% yield from the reaction of indigo with (±)-epichlorohydrin at 85–87 ˚C for 2 h (Scheme 3). Subsequent optimisation gave **11** in 20% yield using (*S*)-**7** (Table 1, Entry 6).

Table 1. Optimisation studies of the reaction of indigo with epichlorohydrin (**7**), and other (halomethyl)oxiranes.

	Halide	Time	Additives	**8** (%)	**9** (%)	**10** (%)	**11** (%)	Baseline material (%mass)
1	Cl (**7**)	30 min		44	30	21		
2	Cl†	1 h	3 Å sieves	41	9	[b]	[b]	30
3	Cl†	2 h	3 Å sieves	44	6	7	6[c]	46
4	Cl†	30 min	3 Å sieves	43	17	8		9
5	Cl	30 min	KI[a]	5	[b]			84
6	Cl	30 min		41	30		20	
7[d]	Cl	30 min		32	21	6		
8	Br	30 min		84				
9	OTs	10 min		76				

[a] 0.1 eq. (based on electrophile). [b] Detected by ESI-MS/TLC analyses of the crude mixture, but not isolated. [c] See Scheme 3. Entry 1 describes outcomes as illustrated in Scheme 2. [d] Reaction performed using acetonitrile as solvent at reflux. † racemic chloride used.

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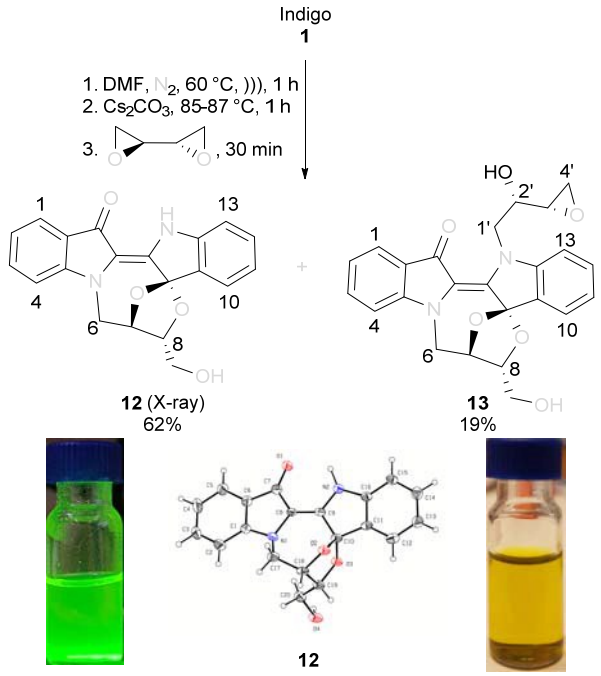


Scheme 4. One-pot cascade reaction of indigo with (2*S*,2'*S*)-2,2'-bioxirane to produce compounds **12** and **13**, and the ORTEP drawing of compound **12**. Below compound **12** is a picture of a solution in acetone, showing its fluorescence under UV light (365 nm); a solution of **13** in dichloromethane (below) shows its colour under ambient light.

HRESI mass spectral analysis revealed the incorporation of both two glycidyl subunits and a carboxylate fragment, evidenced by a peak assigned as $[M+H]^+$ at *m/z* 419.1257, corresponding to the molecular formula $C_{23}H_{18}N_2O_6$. Analysis of the ^{1}H-spectrum of **11** revealed a methine resonance at 5.49 ppm assigned to H2', which showed correlations in the gCOSY spectrum with resonances at 5.20, 4.30, 4.28 and 4.11 ppm assigned to H3'a, H3'b, H1'a and H1'b of the cyclic carbonate pendant, respectively. X-ray crystallographic analysis confirmed the structure of **11**, as well as the relative diastereomeric configuration of the three stereogenic carbons.

In an extension of these oxirane reactivity studies, a mixture of indigo and (2*S*,2'*S*)-2,2'-bioxirane[15] was reacted for 30 min, and upon workup gave a complex mixture, from which two major products (**12** and **13**, Scheme 4) were isolated in a combined 81% yield. Interestingly, both were determined to be products of *N*,*O*'-spirocyclisation, with no *N*,*N*'-cyclised products observed. Both compounds were also determined to be modestly photosensitive to ambient light in solution.

The substituted spiro-dioxolane **12** was isolated as bright red-orange plate crystals in 62% yield, and in solution showed brilliant yellow-green fluorescence under ambient and UV (365 nm) light. The relative stereochemistry of the dioxolane unit was determined by analysis of the ROESY spectrum, which revealed strong through-space correlations between the resonances at 4.74 and 5.00 ppm, assigned as protons H8 and H7, respectively, consistent with a *cis*-arrangement of the C6-C7 bond and the hydroxymethyl pendant to the dioxolane ring. This configuration was confirmed by X-ray crystallographic analysis, and the absence of peak doubling in the ^{13}C spectrum indicated only one diastereomer. The stereochemical purity of the product moreover confirms that a potential Payne rearrangement of the α-hydroxyepoxide resulting from ring-opening[16] – which would produce the corresponding *trans*-dioxolane – had not occurred, and the stereogenic integrity of the precursor bioxirane had been retained.

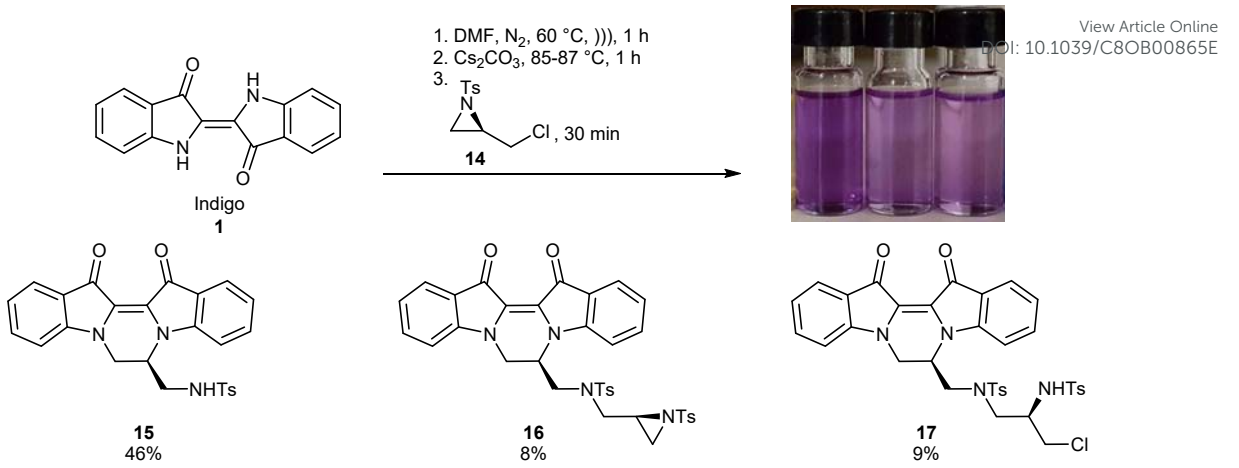
Compound **13**, a *N*-substituted derivative of **7**, was isolated as a pale yellow-green, amorphous solid in 19% yield. Analysis of the ^{1}H-NMR spectrum of **13** revealed geminal proton resonances at δ 2.32 and δ 2.57, assigned as H4'a and H4'b of the pendant oxirane substituent, which TOCSY experiments revealed to be distinct from the *N*,*O*'-spiro tether. This secondary bioxirane unit was confirmed to be a result of *N*'-alkylation by analysis of the HMBC spectrum, which showed a correlation between the C1' methylene protons and C13a.

Reactions of indigo with *N*-sulfonyl-2-(halomethyl)aziridines, *N*-tosyl-2-phenylaziridine and *N*,*N*'-ditosyl-2,2'-biaziridine

N-Sulfonylaziridines show comparable reactivity to oxiranes with respect to rates of ring-opening and Lewis acid-mediated 1,3-dipolar cycloadditions.[17] The reactions of both activated and non-activated aziridines have been extensively reviewed.[18-20] It was anticipated that the use of the corresponding *N*-sulfonyl-2-(halomethyl)aziridines would provide similar outcomes when reacted with indigo to those obtained from reactions with 2-(halomethyl)oxiranes, to give spiro-oxazolidine-containing heterocycles, therefore a small series of simple aziridine precursors was investigated.[21,22] Aziridinyl chloride **14** (which until now had not been synthesised chirally) was prepared conveniently from (*S*)-epichlorohydrin **7** in 64% yield over three steps (see experimental section for details). This was then reacted with indigo under the same optimised conditions as for epichlorohydrin, affording the *N*,*N*'-cyclised product **15** in 46% yield as a glassy blue solid (Scheme 5), in addition to a mixture of several indigo adducts which proved inseparable by flash chromatography, or fractional crystallisation. Subsequent preparative RP-HPLC separation of this fraction afforded compounds **16** and **17** in 8% and 9% yield, respectively, in addition to a fraction deemed to contain a variety of polymeric indigo adducts. As with their oxygenated analogue **8**, solutions of compounds **15**-**17** showed characteristic dark-purple colouration in a variety of solvents in ambient light.

It was anticipated that replacement of the chloro with a bromo leaving group would lead to greater selectivity toward cyclic products **15**-**16**, however the combined high reactivity of this aziridinyl bromide, and the demonstrated nucleophilicity of the liberated sulfonamide, led to uncontrollable polymerisation over longer reaction times. Optimal conditions were established by reacting indigo with (±)-*N*-tosyl-2-(bromomethyl)aziridine[22] for 5 min, to afford (±)-**15** in 52% yield, and a complex mixture of polymeric materials. Moderation of the sulfonyl activator to the smaller *N*-(methanesulfonyl)aziridine dramatically increased the sensitivity of the product heterocycles, producing only uncharacterisable polymeric baseline materials.

To determine whether homocyclisation would occur in the absence of a halomethyl tether, indigo was alkylated with (±)-



Scheme 5. Synthesis of (tosylamidomethyl)dihydropyrazinodiindoles **15**-**17** from the reaction of indigo with aziridinyl chloride **14**. Solutions of compounds **15**, **16**, and **17** (left to right) in dichloromethane are pictured, showing their colour under ambient light. Although compound **16** was synthesised on multiple occasions, its successful isolation from the large pool of additional material (including polymers) was achieved only once.

N-tosyl-2-phenylaziridine (**18**), and yielded exclusively acyclic adducts **19** and **20** in a combined yield of 98% (Scheme 6).

The mono-alkylated indigo derivatives **19** and **20** were isolated as intense, dark-blue powders in 62% yield and 36% yield, respectively, resulting from either terminal (**19**) or benzylic (**20**) ring-opening of the aziridine. Interestingly, despite the proximity of the nucleophilic sulfonamide to the adjacent carbonyl, no evidence for homocyclisation of either **19** or **20** was observed, even upon prolonged heating under basic conditions, supporting the hypothesis that further cyclisation under these conditions is dependent on the presence of suitably-electrophilic functionalities on the *N*-alkyl substituent.

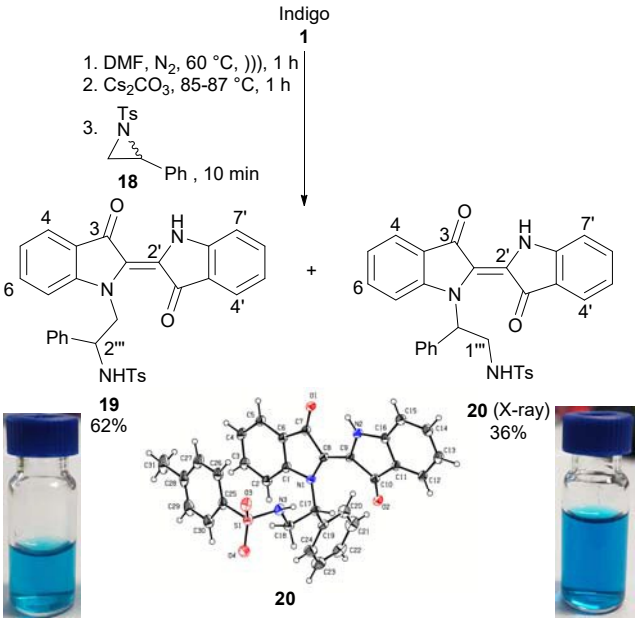
We have recently utilised *N*,*N*'-ditosyl- and *N*,*N*'-diBoc-2,2'-biaziridine (derived from either D- or L-tartaric acid) as convenient precursors to small libraries of enantiopure vicinal diamines and imidazolidinones by ring-opening using a diverse range of nucleophiles.[23-25] Using the indigo anion as a 1,4-di-nucleophile, the reaction of (2*R*,2'*R*) *N*,*N*'-ditosyl-2,2'-biaziridine with indigo afforded the diazepinodiindole **21** in excellent yield (Scheme 7).

Compound **21** was isolated as a dark blue powder in 93% yield, which showed intense deep-purple colouration in dichloromethane solution. Analysis of the ^{13}C-NMR spectrum revealed a pair of carbon resonances at 180.8 and 180.9 ppm, suggesting both carbonyl units of the parent indigo had remained intact. Analysis of the ROESY spectrum revealed a through-space correlation between the H6 and H7 proton resonances at δ 4.95 and 3.62 ppm, suggesting a *cis* orientation, inferring that epimerisation of the parent aziridine had been avoided under these conditions.

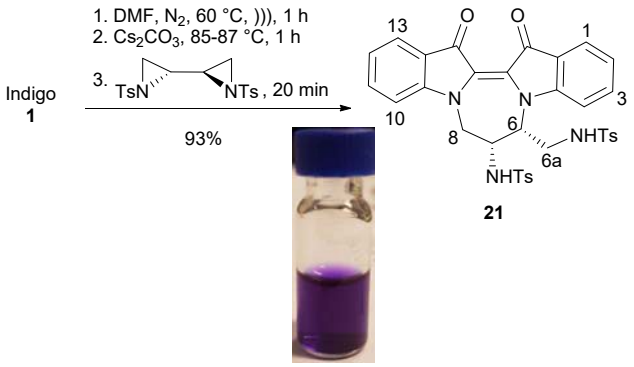
Mechanistic discussion

Reactions of indigo with (halomethyl)oxiranes and 2,2'-bioxirane

Epihalohydrins in polar aprotic solvents undergo preferential nucleophilic attack by ring-opening prior to halide displacement,[26,27] with epoxide ring-opening being up to 200 times more favourable than direct S_N2 attack at the halide. Therefore, the proposed mechanisms for these reactions considered the enthalpic benefit of ring-opening as the



Scheme 6. Synthesis of acyclic aziridine adducts **19** and **20** from indigo by either terminal (**19**) or benzylic (**20**) ring-opening, and ORTEP drawing of benzylic product **20**. Adjacent to each is depicted a solution in acetonitrile of **19** (left, below) and **20** (right, below).



Scheme 7. Reaction of indigo with (2*R*,2'*R*)-2,2'-biaziridine to give compound **21**. Below the structure is pictured a solution of **21** in dichloromethane.

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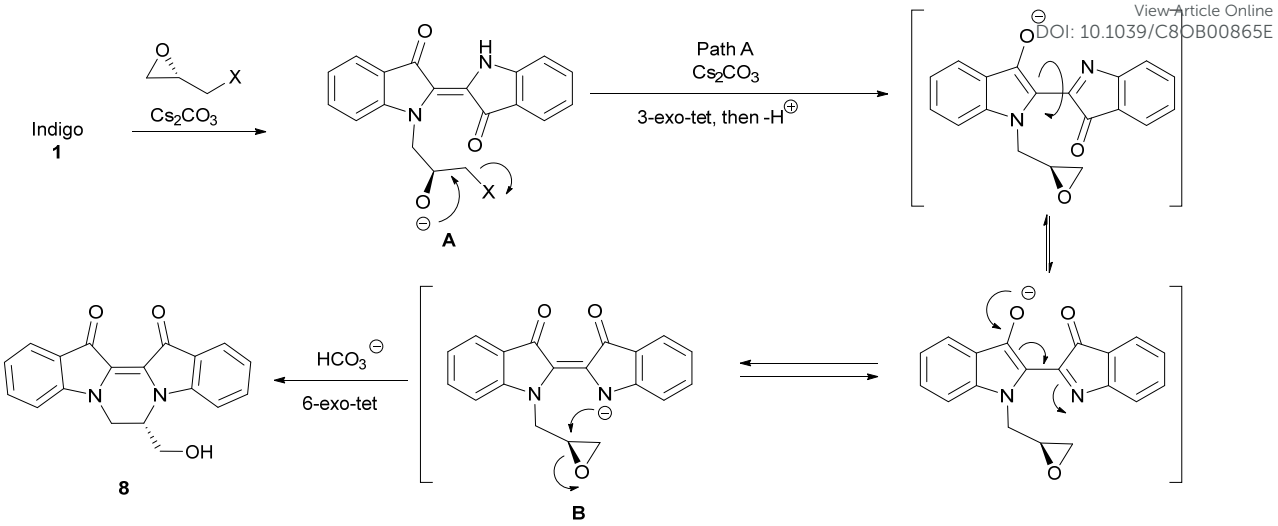
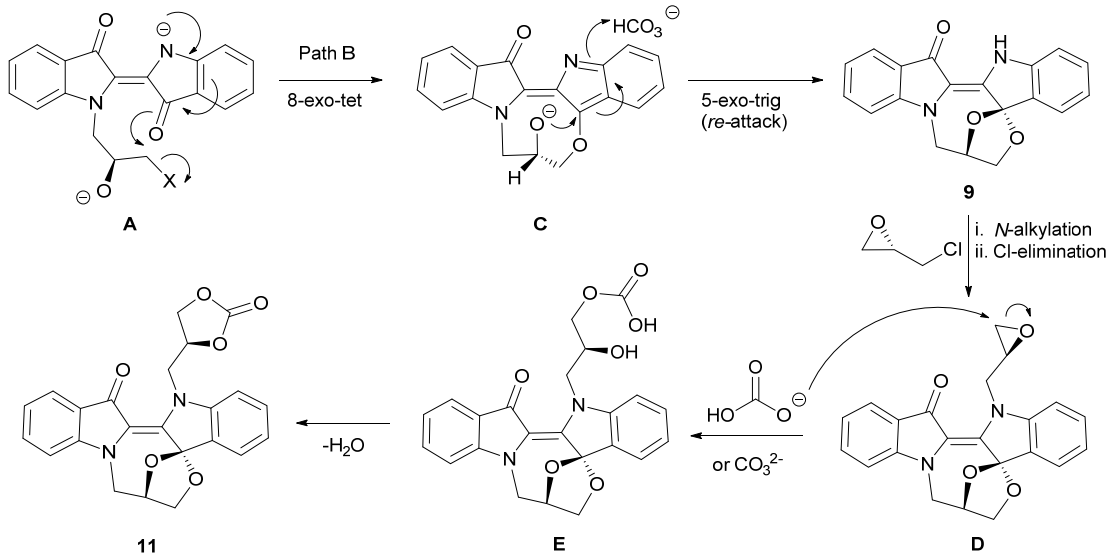


Scheme 8. Proposed mechanism for the formation of 8 from indigo and (S)-epichlorohydrin. Cesium counter-ions omitted for clarity.

energetic driving force for subsequent cyclisation reactions with indigo (Scheme 8). It was observed that moderation of the leaving group from Cl to Br suppressed spiroketalisation, suggesting that the identity of the halide plays a key role in determination of the mechanistic pathway of the reaction. It is proposed that nucleophilic ring-opening of the (halomethyl)oxirane by indigo produces the key intermediate A, from which Path A (Scheme 8) and Path B (Scheme 9) diverge.

Compound **8** is derived from Path A (Scheme 8), where intermediate A undergoes halide elimination by the liberated alkoxide, to reform the epoxide. Prototropic tautomerisation about the indigo double bond occurs readily in the presence of cesium carbonate,[6] which subsequently affords the stabilised cis anion B, and a 6-exo-tet displacement of the more-substituted carbon of the epoxide results in inversion at C6 and formation of the dihydropyrazinodiindole **8**. This process would become more favourable with better leaving groups, as reflected by the increase in yield from 44% to 84% by replacement of X=Cl with X=Br.

It is proposed that spiro-oxazocinodiindoles **9**-**11** are derived from intermediate A where it instead follows Path B (Scheme 9), where the chloride is eliminated by the oxygen of the indigo core *via* a dearomative 8-exo-tet cyclisation to forge the semi-rigid 8-membered ring of intermediate C. The rigidity of this macrocycle impedes attack of the pendant alkoxide to the adjacent indoleninyl C3-position, and directs the nucleophile to the *re*-face, thereby defining the C9a stereochemistry of spirocycle **9**. Further alkylation with additional epichlorohydrin may occur to give D, allowing nucleophilic attack by cesium carbonate, to ring-open the epoxide pendant affording intermediate E, which undergoes subsequent dehydration to afford cyclic carbonate ester **11**. There have been several reported examples of direct addition of oxiranes to ketones to



Scheme 9. Proposed mechanism for the formation of compounds **9** and **11** from intermediate A via Path B.

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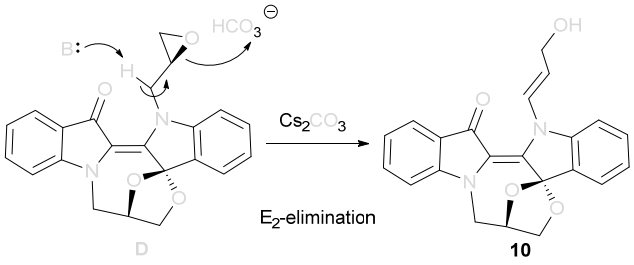


Scheme 10. E2 elimination from intermediate **D** afforded compound **10**.

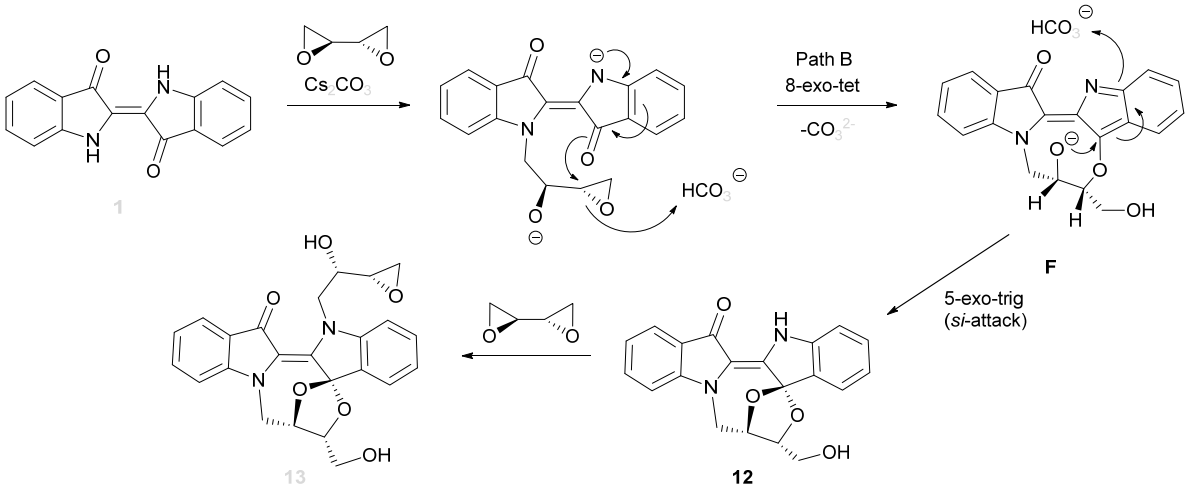
form ketals[28-30] though these typically proceed *via* activation of the oxirane with strong Lewis- or Brönsted acid catalysts. The formation of cyclic carbonates from the addition of CO_2 to oxiranes is also known,[31] however this typically requires the use of high pressures and/or elevated temperature. The low yield (6%) of **11** reported here is attributed to the weakly-nucleophilic character of the carbonate or bicarbonate ions. Compound **10** could similarly be derived from D with cesium carbonate acting as a base, *via* an E2-displacement (Scheme 10). The reaction of indigo (**1**) with (2*S*,2'*S*)-2,2'-bioxirane, where the halide is replaced with a second oxirane unit, results in suppression of Path A. The reaction instead favours Path B (Scheme 11), producing hydroxymethyl-substituted spirocycle **12** by an analogous mechanism to that proposed for the formation of compound **9**, whereby the rigid 8-membered ring (intermediate F) restricts nucleophilic attack at the spiro-carbon to the *si*-face. The stereochemical configuration of the bioxirane moiety is conserved in the *cis*-spirodioxolane product, with the formation of a *trans*-dioxolane *via* a Payne rearrangement not observed under these conditions.[16] Subsequent *N*-alkylation of **12** with a second unit of the bioxirane material affords compound **13**.

Reactions of indigo with aziridines

There was no evidence of spiro-oxazolidine formation from reactions of indigo with aziridine-containing heterocycles. This is largely due to the requirement of strong *N*-activating groups, which both stabilise the liberated heteroanion and provides considerable steric encumbrance to *O*-displacement of the halide. Reactions with (halomethyl)aziridines likely follow an analogous pathway to that in Scheme 8, where ring-closure leads to *N*-alkylindigo intermediate G which undergoes prototropic tautomerisation of the central bond, and *N*-alkylation to afford compound **15** (Scheme 12). Subsequent alkylation of the sulfonamide with excess chloroaziridine **14** affords halosulfonamide **17**, which in the presence of base undergoes a 3-exo-tet cyclisation to afford aziridine **16**.

Compounds **19** and **20** (see Scheme 6, *vide supra*) are the result of direct nucleophilic attack of the indigo nitrogen on a single unit of 2-phenylaziridine, with the absence of a leaving group quickly terminating the cascade process. Interestingly, the two represent differing site-selectivities for nucleophilic attack, where ring-opening at the less-hindered position is favoured over benzylic ring-opening in a 1.7:1 ratio. Preferential electrophilicity of the benzylic position of *N*-tosyl-2-arylaziridines has been previously reported in the presence of Lewis acids eg. $BF_3.OEt_2$, or Pd(II) complexes, resulting in spontaneous ring-opening to externally-stabilised 1,3-dipoles – under such conditions, the sulfonamide-Lewis acid complex bears a formal negative charge, and a benzylic carbocation is generated.[32-34] In the absence of a Lewis acid, the 32% yield of the benzylic product **20** is attributed to the minor steric effect of the phenyl substituent providing only modest site-selectivity, leading to a mixture of both terminal and benzylic adducts.

In our hands, *N*-substituted-2,2'-biaziridines have been shown to undergo facile, sequential ring-opening reactions at the less-hindered C1 and C4 positions, with mono-nucleophiles.[23-25] Contrary to these previous reports, the use of indigo as a di-nucleophile (Scheme 14), results in biaziridine exclusively undergoing 1,3-nucleophilic di-ring-opening, driven by the formation of the stable 7-membered diazepinodiindole **21**, with the high yield (93%) suggesting this to be a highly favourable process. Presumably, the reason for this contrary site-selectivity – via a 1,3-di-alkylation rather than 1,4-di-ring-opening – is the flexibility of the mono-ring-opened chain, whereby steric repulsion between the indigo core and the tosyl activator positions the pendant aziridine distally from the *N*-nucleophile, restricting attack at the terminal C4 carbon. Though macrocyclisation of indigo toward 7 and 8-membered rings has



Scheme 11. Proposed mechanism for the formation of hydroxymethylspirodioxolanes **12** and **13** from indigo via intermediate **F**. Cesium counterions are omitted for clarity.

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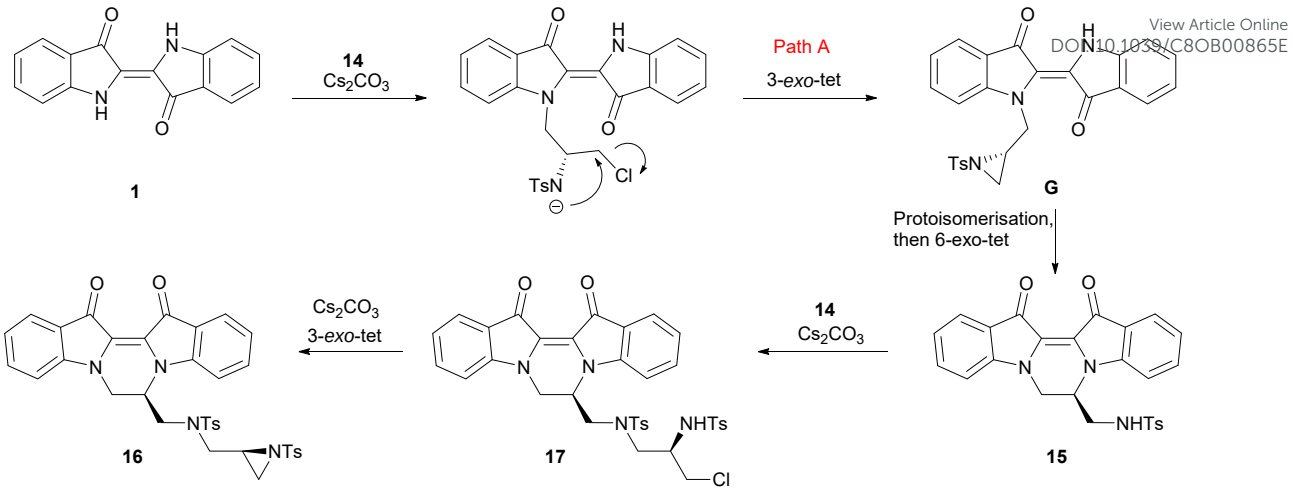


Scheme 12. Formation of pyrazinodiindoles **15**-**17** from the reaction of indigo with (halomethyl)aziridines via Path A.

been reported previously in low yield with alkyl dichlorides,[35] evidently this steric effect provides sufficient impedance to the formation of diazocinodiindole **22** that this otherwise-typical 1,4-di-ring opening pathway is not observed, and the more entropically-favourable heptacycle is exclusively obtained.

The reactions of indigo with strained-ring electrophiles (both oxiranes and aziridines) are scalable up to at least half-gram quantities. In comparison to our previous experiences of the chemistry of indigo (see Scheme 1), π-nucleophiles invoke cascade reactions by nucleophilic attack at the indole C2 with *N*-allyl substituents,[5,7] or ring-expand the biindole nucleus to indolo-naphthiridinones with *N*-propargyl substituents,[6] while the strained-ring electrophiles explored in this study have largely retained the integrity of the indigo core, allowing its integration of new spiro-heterocyclic frameworks. Importantly, these reactions proceed with the stereochemical identity of the parent oxiranes and aziridines intact, suggesting that these cascading processes are the result of sequential nucleophilic displacements, rather than radical chain-reactions.

The formation of both substituted and non-substituted epoxy-oxazocinodiindolones **9**-**13** is unprecedented, and represents the first report of these poly-heterocyclic systems. Importantly, these complex multicyclic frameworks can be selectively synthesised in one pot and in good yields from the exceptionally-cheap starting material indigo. Also of note are instances where cyclisation occurs between the indigo nitrogen

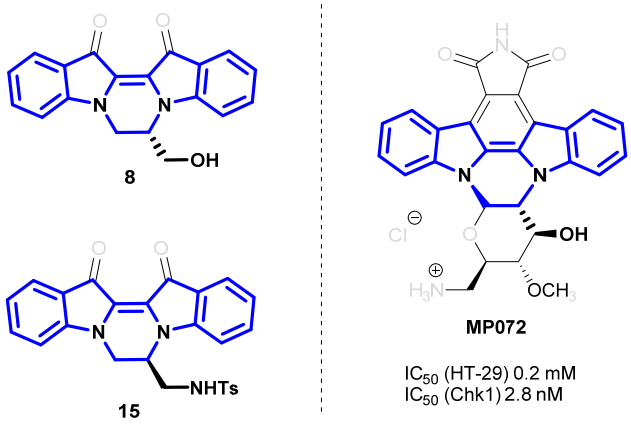
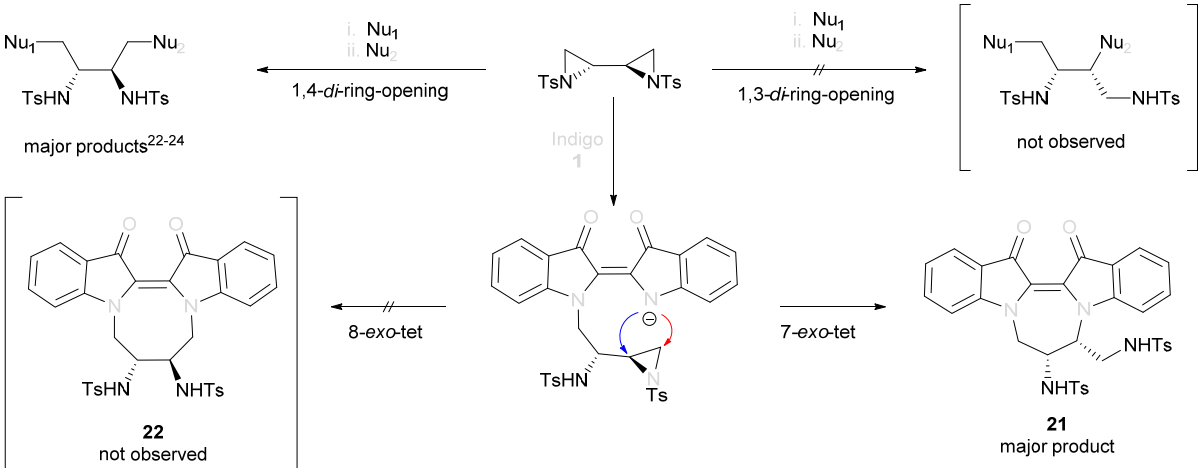


Figure 1. Dihydropyrazinodiindoles **8** and **15** synthesised from indigo, and Staurosporine analogue MP072, a potent checkpoint kinase inhibitor with activity against the HT-29 (colorectal adenocarcinoma) cell line.[41]



Scheme 13. Comparison of reaction outcomes between previous reports,[23-25] and those reported here. Sequential 1,3-di-ring-opening of biaziridine affords diazepinodiindole **21**, rather than the anticipated diazocinodiindole **22**.

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atoms, affording the core dihydropyrazinodiindole scaffold of staurosporine analogue MP072 (Figure 1) in a single step. The presence of malleable functional groups such as hydroxyl- and indolyl-functionalities could allow for the incorporation of these heterocycles into other complex architectures, allowing structural diversification over two steps. The encouraging bioactivities of these scaffolds demonstrate these to be new and effective starting points for the design and synthesis of new biologically-active small molecules. The unique absorptive and fluorescent properties of these and other indigo derivatives may also warrant further investigation – specifically into their materials chemistry applications, as has been previously noted.[36-40]

Biological Testing

Given the promising antiplasmodial potential of the indigo core (see Scheme 1),[6,7] indigo derivates **8**,**11**,**13**, **15**-**17**, and **19**-**21** were subjected to *in vitro* antiplasmodial testing against the malarial parasite *Plasmodium falciparum* (Table 2); compounds **9** and **10** were also tested but they were inactive. All tested compounds containing either the *N*,*N*'-cyclic scaffold (**8**, **15**-**17**, and **21**) or the mono-*N*-substituted indigo scaffold (**19** and **20**) revealed notable antiplasmodial activity in the micromolar range against both the drug-susceptible 3D7 and drug-resistant Dd2 strains of *P. falciparum*, sufficient for each to be considered potential leads for further development. In comparison with the previous lead compound **2** (IC_{50} 0.85 μM), (±)-**8**, (*S*)-**8**, and (*R*)-**8** displayed exceptional and equivalent potencies (3D7 IC_{50} 0.077, 0.1, and 0.09 μM, respectively), showing significantly greater activity than **2**.[6] Compounds of subset **8** in particular are more than an order of magnitude more potent than all other compounds disclosed herein. Importantly, these new compounds show diminished cytotoxicity toward the human HEK 293 cell line, with SI (selectivity index; 3D7/HEK293) ratios >10 in most cases, and for the stereoisomers of **8**, all show two orders-of-magnitude-greater activity against *P. falciparum* than the human HEK 293 cell line, with SI values ranging from 98-120. The activity of most compounds against the drug-resistant Dd2 strain followed a similar trend as against 3D7, with IC50 ratios ranging between 1.2 (**11**) and 2.6 (±)-**8** (only the weakly active compound **15** showed an IC_{50} ratio of 4.5, still within the accepted limits of equipotency).

Conclusions

We report here a series of new cascade reactions of the chemically-overlooked dye indigo via base-mediated alkylation with ring-strained electrophiles. The ability of indigo to act as both nucleophile and electrophile throughout the duration of the reaction – combined with the bifunctional activity of epoxides and aziridines – initiates, and then propagates a cascade process, accessing unprecedented polycyclic architectures from simple, one-pot reactions. Importantly, divergent access to a variety of both heteroatom-substituted dihydropyrazinodiindoles and dihydrodiazepinodiindoles *via* *N*,*N*'-cyclisation is possible, as well as the first documented examples of both substituted and unsubstituted oxazocinodiindoles via *N*,*O*'-spirocyclisation. In particular, the potent antiplasmodial activity of some of the synthesised *N*,*N*'-cycles with good selective toxicity warrants further investigation as new leads for the treatment of malaria. That this diverse array of chemical transformations can be achieved from the cheap and readily-available precursor indigo demonstrates the significant potential in the efficient construction of novel fused-heterocyclic frameworks from this multi-faceted natural dye. We are currently pursuing the discovery of the next-generation of cascade reactions of indigo, aiming towards the development of a greater understanding of the cascade chemistry of indigo, to investigate the chemical, biochemical, and photophysical characteristics of these new polycyclic architectures.

Table 2. *In vitro* antiplasmodial and human cell toxicity data of selected indigo derivatives from this work.

Compound ID	IC50 3D7 (nM)	IC50 Dd2 (nM)	IC50 HEK293 (nM)[a]	Dd2/3D7 IC_{50} ratio	SI (3D7/HEK293 IC_{50} ratio)
(±)-8	76.6 ± 4.0	201.7 ± 7.4	7726 ± 467	2.6	101
(*R*)-8	88.6 ± 16.3	164.5 ± 32.2	10708 ± 1090	1.9	120
(*S*)-8	105.6 ± 37.4	195.5 ± 0.2	10361 ± 1028	1.9	98
21	2767 ± 731	4594 ± 1623	14677 ± 2000	1.7	5.3
16	3075 ± 600	3869 ± 2121	66% at 80 μM	1.3	> 11
19	3698 ± 731	6009 ± 2406	IA	1.6	> 21
20	3976 ± 801	8118 ± 55	IA	2.0	> 20
17	4653 ± 2472	8127 ± 5605	IA	1.7	> 17
15	5625 ± 1775	25209 ± 5459	IA	4.5	> 28
13	9411 ± 2082	16076 ± 4192	64% at 80 μM	1.7	> 2
11	9922 ± 3476	21501 ± 5817	IA	2.2	> 8
Artesunate	1.09 ± 0.03	1.74 ± 0.50	71% at 10 μM	1.6	> 2000
Chloroquine	5.81 ± 0.13	94.6 ± 24.9	67% at 40 μM	16.3	> 3000
Dihydroartemisinin	0.40 ± 0.05	0.57 ± 0.08	62% at 10 μM	1.4	> 10000
Puromycin	141.9 ± 12.7	147.4 ± 23.3	1202 ± 3	1.0	8.47
Pyrimethamine	4.00 ± 0.05	IA	73% @ 40 μM	(resistant)	> 1250

[a]Percent inhibition at highest concentration is given if an IC_{50} value could not be calculated due to dose-response curve not reaching full inhibition plateau; IA = inactive (i.e. < 50% inhibition at the highest concentration).

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Conflicts of interest

There are no conflicts to declare.

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