

N,N'-Bis(6^A-deoxy-β-cyclodextrin-6^A-yl)urea as a molecular template in the formation of indigoid dyes

Christopher J. Easton,^{*,†,a} Jason B. Harper^a and Stephen F. Lincoln^b

^a Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia

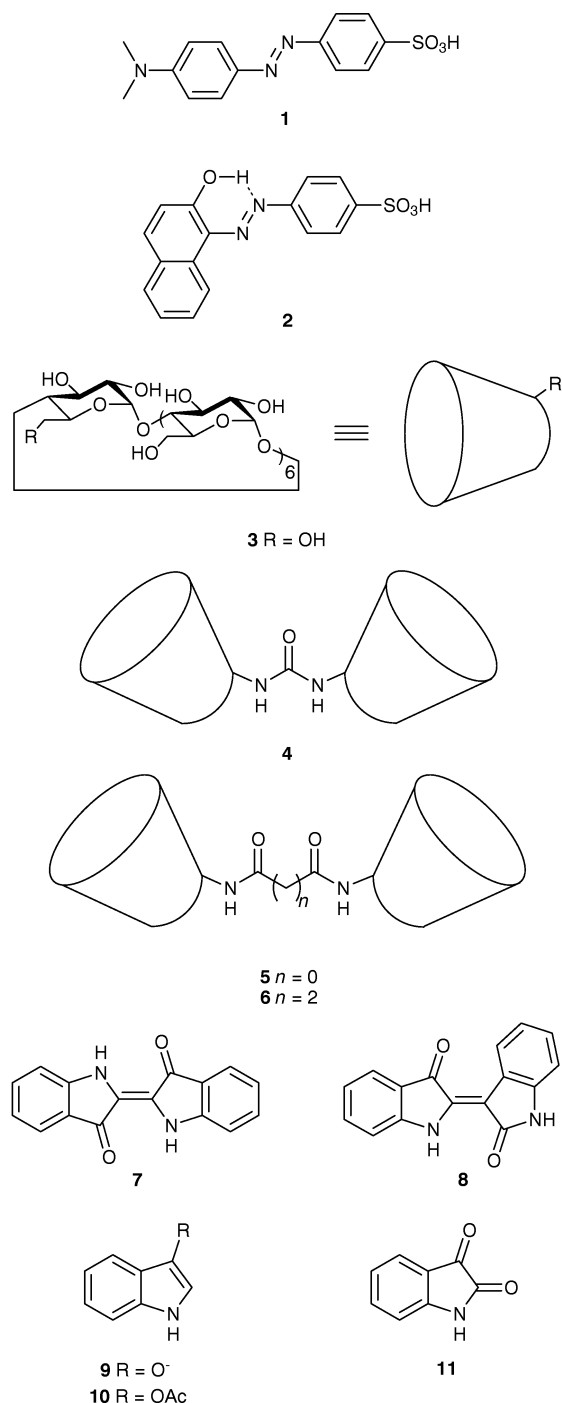
^b Department of Chemistry, University of Adelaide, Adelaide SA 5005, Australia

The title cyclodextrin dimer biases competing reactions of indoxyl anion to give indirubin and indigo against the latter, due to the preferred geometry of alignment of the cyclodextrin annuli.

Linked cyclodextrins have been studied widely due to the cooperative guest binding displayed by the cyclodextrin annuli in such species.^{1–5} Breslow and Chung³ demonstrated that the extent of this cooperativity is dependent on the match of the shape of the guest to the relative orientations of the host cavities. Our studies⁴ of the complexation of the anions of Methyl Orange (**1**) and Tropaeolin 000 No. 2 (**2**) by the cyclodextrins **4–6**⁵ showed maximum binding of the linear dye **1** in the oxalamide **5** and of the non-linear guest **2** in the urea **4**, implying a preferred non-linear orientation of the cyclodextrin annuli in the latter host. The present work involved the exploitation of this observation, using the urea **4** as a molecular template, in the formation of a non-linear product from reagents complexed in the cyclodextrin annuli. This is the first example of the controlled orientation of a coupling reaction by a cyclodextrin dimer. Δ^{2,2'}-Biindoline-3,3'-dione (indigo) (**7**) and Δ^{2,3'}-biindoline-2',3-dione (indirubin) (**8**) form competitively from the oxidative dimerisation of the 1*H*-indol-3-ol anion (indoxyl anion) (**9**) and its condensation with 1*H*-indoline-2,3-dione (isatin) (**11**), respectively.⁶ Isatin (**11**) is formed in the reaction as an oxidation product of indoxyl, indigo (**7**) and indirubin (**8**). Given the shape of the dyes **7** and **8** and the geometry of the cyclodextrin **4**, it was considered that complexation of the reactants **9** and **11** by the cyclodextrin **4** could bias the ratio of dye formation in favour of indirubin (**8**).

To examine this hypothesis, indoxyl anion (**9**) was generated *in situ*, through hydrolysis of the corresponding acetate **10**, in buffered aqueous solutions containing a *ca.* 6 molar excess of isatin (**11**) and either no cyclodextrin, β-cyclodextrin (**3**) or one of the linked species **4–6**. By comparison with the concentration of the linked species **4–6**, approximately double the concentration of β-cyclodextrin (**3**) was used, in order to achieve a cyclodextrin annuli concentration *ca.* 130 times that of the acetate **10**, in each case. The reactions were studied at pH 10.0, 10.5 and 11.0. Basic conditions are required for hydrolysis of indoxyl acetate (**10**) to indoxyl and deprotonation of indoxyl to give the anion **9**, but side reactions of indoxyl are prevalent below pH 10^{6,7} and hydrolysis of isatin (**11**) is the dominant reaction of that species in phosphate buffer at or above pH 11.⁸ Reactions were stopped by acidification of the mixtures, then the products **7** and **8** were extracted into chloroform and the extracts were analysed using HPLC.

The results of these experiments are shown in Table 1. They show that an effect of each of the cyclodextrins **3–6** is to decrease the yields of both indigo (**7**) and indirubin (**8**). This is as expected, since complexation of the reactants **9** and **11** by the large excess of cyclodextrin annuli reduces their opportunity for productive collisions and favours their mono-



† E-mail: easton@rsc.anu.edu.au

Table 1 Yields of indigo (7) and indirubin (8) from reactions of indoxyl anion (9) and isatin (11)

Cyclodextrin	Percentage yields of indigo (7) and indirubin (8) as the ratio		
	pH 10.0	pH 10.5	pH 11.0
—	16 : 13	41 : 38	47 : 12†
3	2.5 : 2.5	8.5 : 9.0	9.5 : 12
4	0.03 : 1.0	0.4 : 7.5	2.5 : 17
5	0.2 : 0.6	9.0 : 6.5	15 : 14
6	0.5 : 0.7	5.5 : 8.5	6.0 : 6.5

† The high ratio in this case is consistent with rapid hydrolysis of isatin (11) at the high pH. This is slowed through complexation by the cyclodextrins 3–6, so the product ratio is not as high from reactions in the presence of those species.

meric reactions, at the expense of their second-order reactions to give the dyes 7 and 8. By comparison with the other cyclodextrins 3, 5 and 6, the cyclodextrin 4 has a marked effect on the ratio of formation of indigo (7) and indirubin (8). For example, at pH 10, use of the cyclodextrin 4 affords the dyes 7 and 8 in a 1 : 33 ratio. By contrast, the reactions carried out in the absence of that host give ratios of the products 7 and 8 ranging from 4 : 1 to 1 : 3, with most being approximately 1 : 1.

Any interpretation of the results shown in Table 1 must account for the particular effect of the cyclodextrin 4, which is not to produce a higher yield of indirubin (8), but to reduce sharply the amount of indigo (7) that forms. This decrease shows that most of the indoxyl anion (9) must be complexed by the cyclodextrin 4, and in an orientation that does not allow the oxidative dimerisation, which occurs in free solution to give indigo (7). However, the complexed anion 9 must still be able to react with isatin (11) to form indirubin (8), as it does in solution. It is reasonable to assume this involves isatin (11) that is complexed, since the more hydrophilic anion 9 is complexed under these conditions.¹ This assumption is supported by separate observations that the cyclodextrins 3–6 reduce the rate of degradation of isatin (11) at high pH, presumably by limiting hydrolysis through complexation.

The most probable orientation of the anion 9 in a cyclodextrin annulus is with the enolate portion protruding from the narrow end, delineated by the primary hydroxyl groups (Fig. 1). This is consistent with the antiparallel alignment of

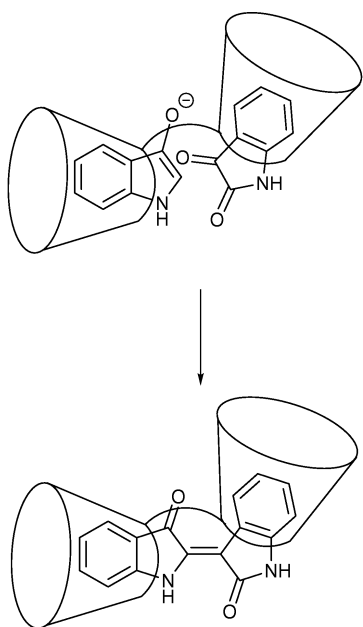


Fig. 1 Representation of the effect of the cyclodextrin 4 as a template for the condensation of indoxyl anion (9) with isatin (11)

the dipole moments of the hosts and guests in cyclodextrin host–guest complexes.⁹ It also accounts for the greatly reduced yields of indigo (7) from the reactions carried out in the presence of the dimer 4, compared to those involving any of the cyclodextrins 3, 5 or 6. With the orientation of the anion 9 shown in Fig. 1, the enolate is most shielded when complexed by the dimer 4, but in the opposite orientation; each of the cyclodextrins 3–6 would be expected to have a similar effect on the yield of indigo (7). Oxidative dimerisation of the complexed anion 9, to give indigo (7), is disfavoured by the dimer 4, presumably as a result of the unsuitable geometry of alignment of the cyclodextrin annuli. At the same time, the geometry of the cyclodextrin dimer 4 allows reaction of the anion 9 with complexed isatin (11) to give indirubin (8) (Fig. 1). Therefore, while the cyclodextrin 4 acts to limit the formation of indigo (7) rather than promote the production of indirubin (8), it is clearly acting as a template for the preassembly of reagents, to bias competing reactions of indoxyl anion (9).

Experimental

β-Cyclodextrin (3) was the generous gift of Nihon Shokuhin Kako Co., while the cyclodextrin dimers 4–6 were synthesized as previously reported.⁵ All cyclodextrins were dried to constant weight *in vacuo* over phosphorus pentoxide prior to use. Indigo (7) and indoxyl acetate (10) were purchased from Sigma–Aldrich Chemical Company Inc. and isatin (11) was purchased from BDH Ltd. Indirubin (8) was prepared as described previously.⁶ Borate buffers (pH 10.0 and pH 10.5, 0.1 mol dm^{−3}) and phosphate buffer (pH 11.0, 5.0 × 10^{−2} mol dm^{−3}) were prepared using standard procedures.¹⁰

In a typical procedure, indoxyl acetate (10) (1.7 mg, 9.7 μmol) and isatin (11) (8.5 mg, 59 μmol) were dissolved in 10.0 cm³ of the appropriate buffer. Samples of this solution (0.10 cm³) were immediately added to solutions containing either no cyclodextrin, β-cyclodextrin (3) (ca. 14 μmol) or one of species 4–6 (ca. 7.0 μmol), in 0.90 cm³ of the appropriate buffer. The reactions at pH 10.0 and 10.5 were stirred for 16 h, then quenched with acid. The reactions at pH 11.0 were quenched after 15 min. The product mixtures were extracted with chloroform and the extracts were analysed using HPLC on an Alltech Econosil column (5 μm silica, 4.6 × 250 mm), eluting with acetone–chloroform (5 : 95) and monitoring at 550 nm using a detector calibrated with authentic samples. The results are shown in Table 1 and represent the average of replicate injections of products from at least two reactions. The product yields from replicate experiments differ by less than 15%.

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