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A cyclodextrin-based molecular reactor to template the formation of indigoid dyes

Jason B. Harper,[a,†] Christopher J. Easton[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*

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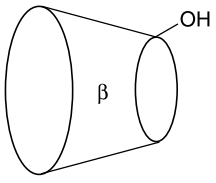
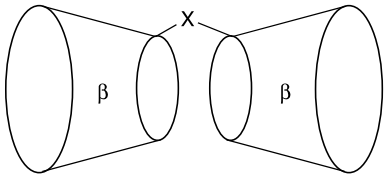
Abstract—*N*,*N*′-Bis(6^{A}-deoxy-β-cyclodextrin-6^{A}-yl)urea behaves as a molecular reactor to bias competing reactions of indoxyl anion and isatin-5-sulfonate in water, to give indigo and indirubin-5′-sulfonate. It appears that the cyclodextrin dimer increases the relative reactivity of the isatin-5-sulfonate, by selectively complexing the reactive form. The molecular host also aligns the isatinsulfonate with indoxyl anion to favour production of indirubin-5′-sulfonate, with the result that the ratio of indigo and indirubin-5′-sulfonate produced is altered by a factor of at least 3500, without a substantial loss of yield.

Cyclodextrins form inclusion complexes with small organic compounds in water and catalyse reactions of the included species.[1–5] In this regard they may be considered enzyme mimics. They have also been exploited as molecular reactors, where they behave as nanoscale containers to control the assembly of reactants and change the outcomes of chemical transformations.[6–17] Probably the most straightforward examples of cyclodextrin-based molecular reactors are those that involve a change in the regioselectivity of reaction as a result of a substrate being included in such a way as to restrict access of a reagent.[6–11] Early work in this area by Breslow et al.,[6–8] showed that cyclodextrins alter the regioselectivity of aromatic substitution. When anisole is included in a cyclodextrin, the *ortho* positions are shielded from chlorination with hypochlorous acid, while the *para* position is still accessible.



β-cyclodextrin (**1**)



2a X = NHCONH
2b X = NHCOCONH
2c X = NHCO(CH$_2$)$_2$CONH

Molecular templates for carbon–carbon bond-forming reactions have also been developed. Imidazole-substituted cyclodextrins have been used to control intramolecular aldol reactions,[12,13] dehydroalanine has been bound to a cyclodextrin to bring about its reaction with indole to give tryptophan,[14] and dipolarophiles have been attached to cyclodextrins to reverse the regioselectivity of cycloadditions with nitrile oxides.[15,16] In another example, we exploited the cyclodextrin dimer **2a** as a molecular reactor, to bias the competition between oxidative dimerisation of the 1*H*-indol-3-ol anion (indoxyl anion) (**3**) and its condensation with 1*H*-indoline-2,3-dione (isatin) (**4a**), to give $\Delta^{2,2'}$-biindoline-3,3′-dione (indigo) (**5**) and $\Delta^{2,3'}$-biindoline-2′,3-dione (indirubin) (**6a**), respectively (Scheme 1).[17] Our rationale for using this template was based on the particularly strong binding of Tropaeolin 000 No. 2 (**7**) by the dimer **2a**,[18] and the similarity between the orientation of the dimer **2a** required for efficient binding of the guest **7** and that necessary to favour reaction of indoxyl anion (**3**) with isatin (**4a**) to give indirubin (**6a**) (Fig. 1). In the event, addition of the dimer **2a** changed the ratio of formation of indigo (**5**) to indirubin (**6a**) to ca. 1:30, from ca. 1:1 in either the absence of a cyclodextrin or in the presence of the host **1** (Table 1). The cyclodextrin dimers **2b** and **2c** bound the Tropaeolin **7** much less effectively and they had little effect on the ratio of formation of the indigoid dyes **5** and **6a**.

Unfortunately the use of the cyclodextrins was associated with very substantial decreases in the combined yields of the dyes **5** and **6a**, from 29% in the absence of a cyclodextrin, to 5% in the presence of β-cyclodextrin (**1**) and ca. 1% in the presence of the dimer **2a**. This can

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* Corresponding author. Tel.: +61 2 6125 8201; fax: +61 2 6125 8114; e-mail: easton@rsc.anu.edu.au
† Present address: School of Chemical Sciences, University of New South Wales, Sydney NSW 2052, Australia.

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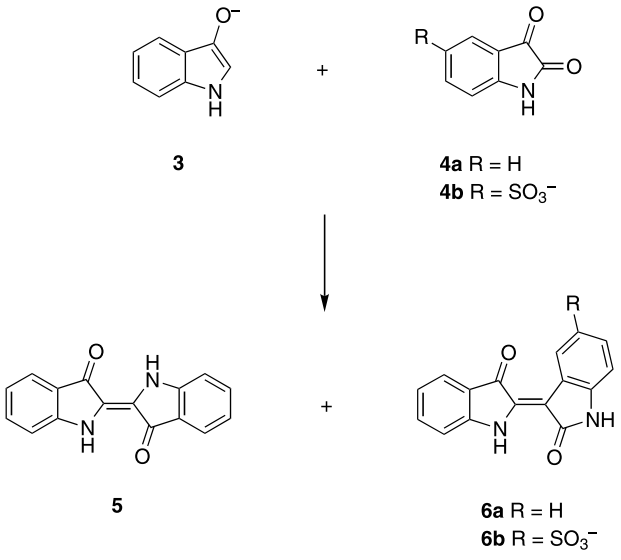
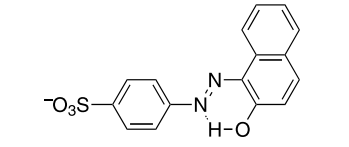


Scheme 1.



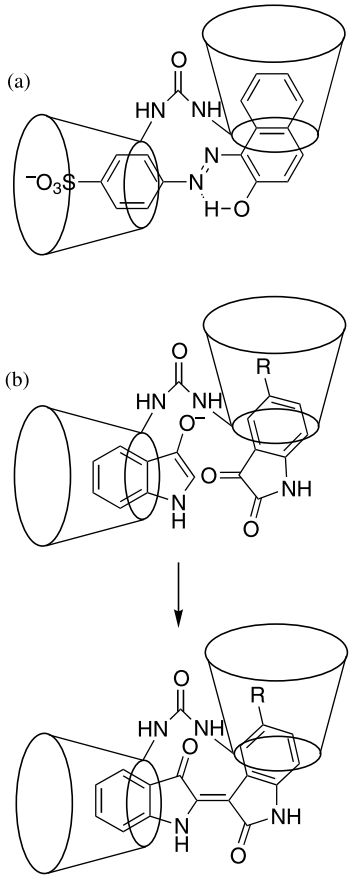


Figure 1. Schematic representation of the orientation of the cyclodextrin dimer **2a** required to (a) bind the dye **7** and (b) template the synthesis of indirubin (**6a**) and the sulfonate **6b**.

Table 1. Percentage yields of the dyes **5** and **6a** from reactions of indoxyl anion (**3**) and isatin (**4a**) at ambient temperature in 0.01 M borate buffer at pH 10.0[17]

Cyclodextrin	Product	
	5	**6a**
–	16	13
1	2.5	2.5
2a	0.03	1.0
2b	0.2	0.6
2c	0.5	0.7

be attributed to the inclusion of the substrates **3** and **4a** within each of the cyclodextrins increasing their effective steric bulk and therefore decreasing the frequency of their productive collisions to give the dyes **5** and **6a**. At the same time the rate of hydrolytic decomposition of the substrates **3** and **4a** is largely unaffected, so these processes become more dominant. To overcome this, it is necessary to avoid decreasing the reactivity of one or both of the substrates **3** and **4a** towards formation of the dyes **5** and **6a** as a result of complex formation and we are now able to report that this has been accomplished by using 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonate (**4b**) in place of isatin (**4a**). In this case the urea-linked cyclodextrin dimer **2a** templates the reaction to give indirubin-5′-sulfonate (**6b**), without significantly reducing the yield. Further, the product bias displayed by the cyclodextrin **2a** is much greater and changes the ratio of formation of the dyes **5** and **6a** by a factor of at least 3500.

The reactions of indoxyl anion (**3**) and the isatinsulfonate **4b** were carried out as described previously for the non-sulfonated isatin (**4a**).[17] The anion **3** was generated in situ through hydrolysis of the corresponding acetate (1 μM) in 0.01 M aqueous borate buffer at pH 10.0, containing a ca. 6 molar excess of the isatinsulfonate **4b** and either no cyclodextrin, β-cyclodextrin (**1**) (20 μM) or one of the linked species **2a**–**c** (10 μM). After 16 h, the reactions were quenched through acidification, and the mixtures were then partitioned with chloroform. HPLC analysis on an Alltech Econosil column (5 μM silica, 4.6×250 mm), eluting with acetone–chloroform and monitoring at 550 nm using a system calibrated with authentic samples, showed that the organic phase contained all the indigo (**5**) while all the indirubinsulfonate **6b** remained in the aqueous phase. The separate phases were therefore easily analysed using UV spectroscopy, based on the molar extinction coefficients of indigo (**5**) and the indirubinsulfonate **6b** of 14000 and 7890 M^{-1} cm^{-1}, in chloroform and water, respectively, to determine the yields of the dyes **5** and **6b**, and the results are shown in Table 2.

Addition of β-cyclodextrin (**1**) changes the ratio of indigo (**5**) to the sulfonate **6b** produced, from ca. 18:1 to 1:7; that is, by a factor of ca. 120. This effect is most probably related to the fact that in solution the sulfo-

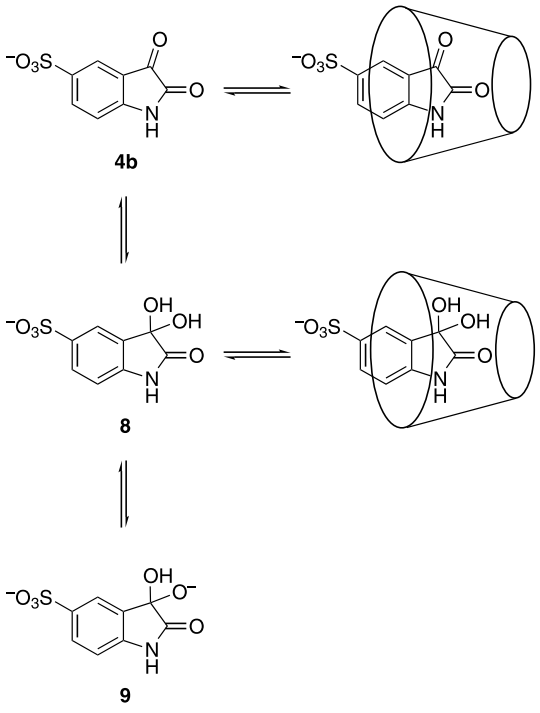
Table 2. Percentage yields of the dyes **5** and **6b** from reactions of indoxyl anion (**3**) and isatin-5′-sulfonate (**4b**) at ambient temperature in 0.01 M borate buffer at pH 10.0

Cyclodextrin	Product	
	5	**6b**
–	25	1.4
1	1.6	11
2a	<0.1[a]	22
2b	1.8	36
2c	6.0	16

[a] Below the detection limit of the method.

nate **4b** exists partially as the hydrate **8**, which has a pK_a of 9.55 and deprotonates to give the anion **9** (Scheme 2).[19] The cyclodextrin **1** appears to complex the isatin **4b** and the corresponding hydrate **8** in preference to the anion **9**. Evidence for this was obtained using ^{1}H NMR spectroscopy.

At neutral pH and below, buffered aqueous solutions prepared using the sodium salt of the sulfonate **4b** (5 mM) showed two discrete sets of resonances in their ^{1}H NMR spectra. These were observed at δ 7.09 (d, $J=7$ Hz, C7-H), 7.95 (s, C4-H) and 7.99 (d, $J=7$ Hz, C6-H), and at δ 7.07 (d, $J=7$ Hz, C7-H), 7.77 (s, C4-H) and 7.81 (d, $J=7$ Hz, C6-H), corresponding to the isatin **4b** and the hydrate **8**, respectively.[19] Integration of these signals shows that the species **4b** and **8** are present in a ratio of ca. 2:1. In the presence of β-cyclodextrin (**1**) (10 mM), but under otherwise identical conditions, the same resonances are observed for the isatin **4b** and the hydrate **8** but in a ratio of ca. 4:1. The cyclodextrin **1** biases the ratio by complexing the non-hydrated form **4b** in preference to the hydrate **8** (Scheme 2).



Scheme 2. Complexation of the isatin **4b** and the corresponding hydrate **8** within the cyclodextrin **1** limits formation of the dianion **9**.

In equivalent solutions buffered at pH 9.0, some peak broadening occurs in the ^{1}H NMR spectra, presumably due to the formation of the anion **9** and rapid interconversion of the indolines **4b**, **8** and **9** on the NMR time-scale. Analogous solutions buffered at pH 9.5 and above gave rise to ^{1}H NMR spectra in which peak broadening is too extensive for it to be practical to assign resonances to individual species. This broadening occurs both in the presence and absence of the cyclodextrin **1**, but the extent of the broadening is less when the host **1** is present.

The effect of the cyclodextrin **1** is difficult to quantify from these results and decomposition of the isatin **4b** at high pH[20] prevents a more thorough analysis. Nevertheless, the broadening in the ^{1}H NMR spectra of solutions containing the cyclodextrin **1** and buffered at pH 10.0 is less than that seen in solutions buffered at pH 9.5 that did not contain any cyclodextrin. This implies that the cyclodextrin **1** increases the pK_a value of the hydrate **8** by more than 0.5 units. This is consistent with complexation of the isatin **4b** and the hydrate **8** in preference to the anion **9**, as might be expected due to the increased ionic character of the latter. There is ample literature precedent for such an effect, since cyclodextrins are known to alter the pK_a values of phenols[21] and benzoic acids[22] through selective complexation of the acid forms.

A consequence of the complexation of the non-hydrated isatin **4b** in preference to the hydrate **8**, and the change in the pK_a value of the hydrate **8**, is that at high pH the cyclodextrin **1** substantially increases the proportion of the isatin **4b** present in the mixture. Since it is this species that reacts with indoxyl anion (**3**) to form the sulfonated indirubin **6b**, the addition of the host **1** increases the amount available so that more of the dye **6b** is produced. A similar effect is observed with each of the dimeric hosts **2b** and **2c**.

Thus, the reactivity of indoxyl anion (**3**) and isatin (**4a**) is reduced by complexation in either β-cyclodextrin (**1**) or the dimers **2b** and **2c**, and the yields of the reactions to give indigo (**5**) and indirubin (**6a**) are reduced as a consequence (Table 1). By comparison, the hosts **1** and **2b**,**c** do not reduce the reactivity of the isatinsulfonate **4b**, at least to the same extent. Any loss of reactivity as a result of inclusion is offset, and may even be outweighed, by selective complexation of the reactive form **4b**. As a result, the cross-coupling of indoxyl anion (**3**) with the isatinsulfonate **4b**, to give the indirubinsulfonate **6b**, is favoured by the enhanced reactivity of the sulfonate **4b** relative to that of the anion **3**. Further, the combined yields of the dyes **5** and **6b** are not significantly decreased (Table 2). The effects are different with isatin (**4a**) and the isatinsulfonate **4b** because only the latter is hydrated in aqueous solutions to any meaningful extent.

In the case of the urea-linked dimer **2a**, superimposed on the effect of the host to increase the proportion of the isatinsulfonate **4b** present in the mixture is a templating effect analogous to that seen in the reactions of indoxyl anion (**3**) with isatin (**4a**). This is apparent from comparison of the effects of β-cyclodextrin (**1**) and the dimer **2a** on the yields of the dyes **5** and **6a** (Table 1), and **5** and **6b** (Table 2). The preferred geometry of alignment of the cyclodextrin annuli of the dimer **2a** further favours formation of the isatinsulfonate **6b** and limits the production of indigo **5** (Fig. 1). The net effect of the molecular reactor **2a** is therefore to change the ratio of formation of the dyes **5** and **6b** by a factor of at least 3500 without a substantial loss of yield.

In conclusion, by using the isatinsulfonate **4b** in place of isatin (**4a**), it has been possible to overcome the loss of yield resulting from the decrease in reactivity associated with inclusion in the cyclodextrin dimer **2a**. As a result the effect of the host **2a** to bias the ratio of formation of indigoid dyes in water at ambient temperature is also greatly magnified. Therefore the dimer **2a** is demonstrated to be a very efficient molecular reactor.

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