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A Rapid and Reliable Assay for Regioselectivity Using Fluorescence Spectroscopy

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Abstract: The first report of a fluorescence-based assay for the direct measurement of the regioselectivity of a reaction is described herein, developed from the desire to construct a quicker analytical method for the determination of the ratio of regioisomers obtained in the tandem hydroformylation/Fischer indole synthesis. The method allows for extremely rapid acquisition times, as the use of crude reaction mixtures is allowed. The assay is also shown to be overall very reliable, tolerating the presence of various functional groups and proceeding on average with a standard error of measurement comparable to that of NMR. As fluorescence is the only requirement for the employment of this analytical method, countless numbers of target-specific assays can undoubtedly be developed based upon this initial finding.

Keywords: fluorescence spectroscopy; hydroformylation; indoles; rapid assay; regioselectivity

Introduction

The ability to quickly and easily monitor the selectivity of any chemical reaction forming a carbon-carbon bond on a small scale *via* spectroscopic means is an indispensable tool to synthetic chemists.[1] The appearance of a number of high-throughput screening methods in the past years has resulted in various possibilities for monitoring chemical reactions and processes.[2] Among these are techniques based on HPLC, colorimetry, MS, IR or fluorescence spectroscopy. However, none of these methods are universally applicable, especially when the screening of catalytic transformations is desired, where many different catalyst:ligand:substrate ratios may be explored producing varying ratios of isomeric products. Examples of this type of strategy have been reported for C–C bond forming reactions such as aldol additions,[3] but examples involving analysis of transition metal-catalyzed processes such as hydroformylation have not been reported. Hydroformylation is one of the most frequently employed homogenously catalyzed processes in the chemical industry and is also a very important tool in organic synthesis.[4] This transformation of an olefin to an aldehyde is extremely versatile as it can be used alone or in sequence with subsequent transformations.[5] A large amount of effort has gone into developing bidentate ligands such as BIPHEPHOS and XANTPHOS designed to address one of the inherent properties of the reaction, namely the production of regioisomeric aldehydes when unsymmetrical olefins are employed as starting materials.[6] Styrenes and their derivatives have proven to be somewhat challenging substrates as they are known to produce mixtures of regioisomers that are less controllable by the addition of a ligand to the reaction.[6b,c,7] When screening ligands on a new substrate, another challenge is frequently encountered in the analysis of the various mixtures of regioisomers generated. Many aldehydes and adducts formed *via* hydroformylation are sensitive and do not lend themselves to even standard methods of analysis such as gas chromatography.

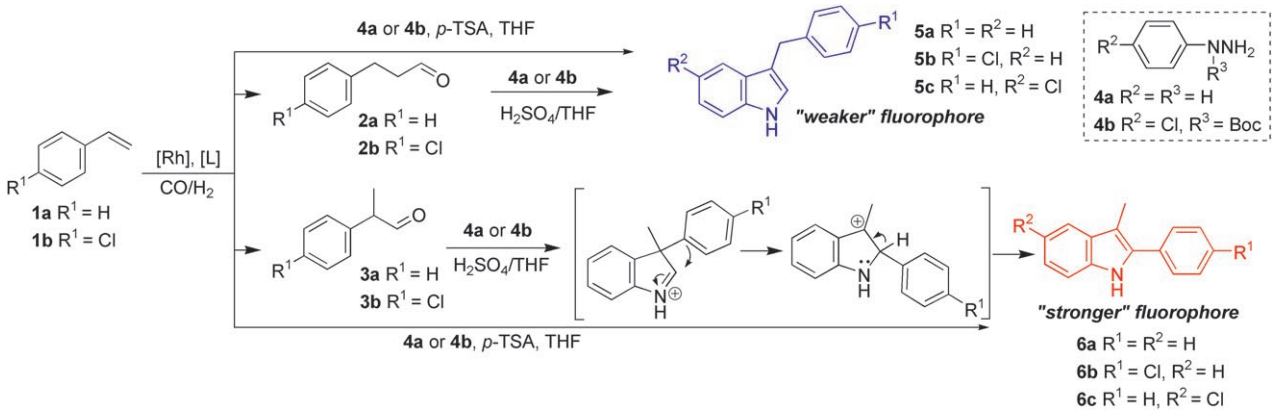
Other techniques requiring large amounts of handling and purification prior to analysis generally prove to be more problematic. Our goal was to bypass these issues standing in the way of a smooth analysis and to develop a method for the rapid and reliable determination of the regioselectivity of the hydroformylation reaction using fluorescence spectroscopy, a technique widely used due to its high sensitivity and simplicity. Here we report a rapid fluorescence assay to monitor and determine the regioselectivity of the sequential Rh-catalyzed hydroformylation/Fischer indole synthesis.

Results and Discussion

Our group has been investigating hydroformylation coupled with tandem/sequential reactions for several years.[5b–e] Much effort has gone into studying the regioselectivity of the hydroformylation under these types of reaction conditions. Recently, significant results have been observed in the sequential hydroformylation/Fischer indole synthesis, where an easy and straightforward synthesis of pharmaceutically active indoles has been discovered.[8] The development of this new analytical method came out of this most recent study, where the need for an improved assay for regioselectivity was demonstrated. Our methodology was inspired by the phenomenon occurring during the indolization of aldehydes with phenylhydrazines, a reaction well explored and recently described by our group elsewhere.[9] In either a stepwise or tandem fashion, the linear *n*-aldehydes **2a,b** arising from styrene hydroformylation proceed to give 3-benzylindoles **5a–c** while the regioisomeric branched *iso*-aldehydes **3a,b** result in 3-methyl-2-phenylindoles **6a–c** as the products of Wagner–Meerwein rearrangement[10] (Scheme 1). As the ratio of *n*-:*iso*-aldehydes is not changed by the indolization reaction conditions, the ratio of the final indole products directly reflects the regioselectivity of the hydroformylation. The increased amount of conjugation present in the rearranged products relative to the 3-benzylindoles ensures that they will exhibit different spectral properties – namely that the highly conjugated system will be a stronger fluorophore than the non-conjugated system – which made this system an ideal one for initial measurements.[11]

Several experiments were performed to test our methodology as a means of determining the regioselectivity of the reaction. To establish a reference, a collection of standard spectra was acquired on known ratios (10:0, 9:1, etc…) of indoles **5a** and **6a**. In all of these cases, the indolization was performed stepwise using $H_2SO_{4(cat.)}$/THF.[9] After removal of the reaction solvent and purification by silica gel chromatography, samples were dissolved to 20 μM in DMSO. In order to ensure equal probability of excitation for both compounds in the mixture ($\lambda_{max\mathbf{5a}} = 284$ nm, $\lambda_{max\mathbf{6a}} = 311$ nm), a wavelength of 275 nm was used since the extinction coefficients of both isomers are roughly the same on this wavelength. The fluorescence spectrum of each mixture was recorded, and the dependence of the ratio of emission intensities at the respective emission maximum as well as the center of spectral mass on the ratio of isomers were calculated. The difference of nearly 30 nm in the emission maxima and the presence of an isosbestic point around 350 nm supported our predictions of two fluorescent regioisomers in the mixture, clearly showing the strong dependence of the fluorescence spectra on the ratio of these regioisomers (Figure 1).

To determine whether the analysis of crude samples was possible, aliquots were simply removed from the reaction and evaporated prior to acquiring the fluorescence spectra. Amazingly, there was no difference observed between crude and pure products, lending greatly to this method's employment as a rapid assay (Figure 2).[12]



Scheme 1. Synthesis of indoles *via* tandem or stepwise hydroformylation/Fischer-indolization.

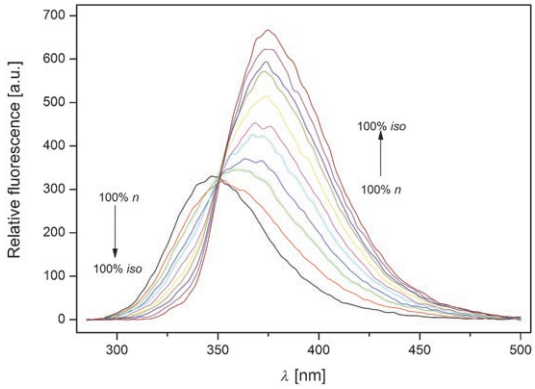


Figure 1. Fluorescence spectra of **5a** and **6a** and their mixtures (**5a**:**6a** = 10:0, 9:1, …, 1:9, 0:10) in DMSO, λ_{ex} = 275 nm, c = 20 μM at 25 °C.

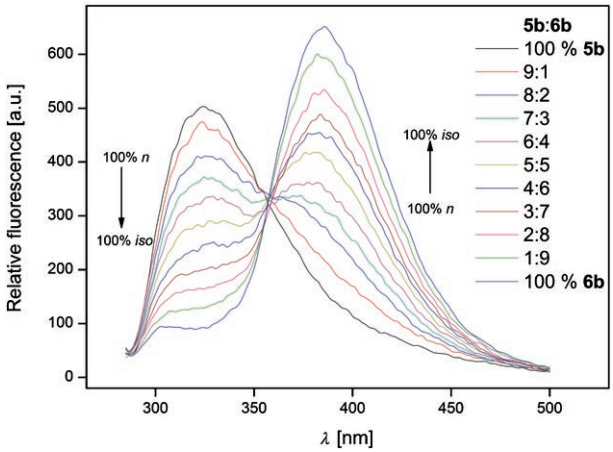


Figure 3. Fluorescence spectra of **5b**, **6b** and their mixtures (λ_{ex} = 275 nm, DMSO, c = 5 μM, 25 °C).

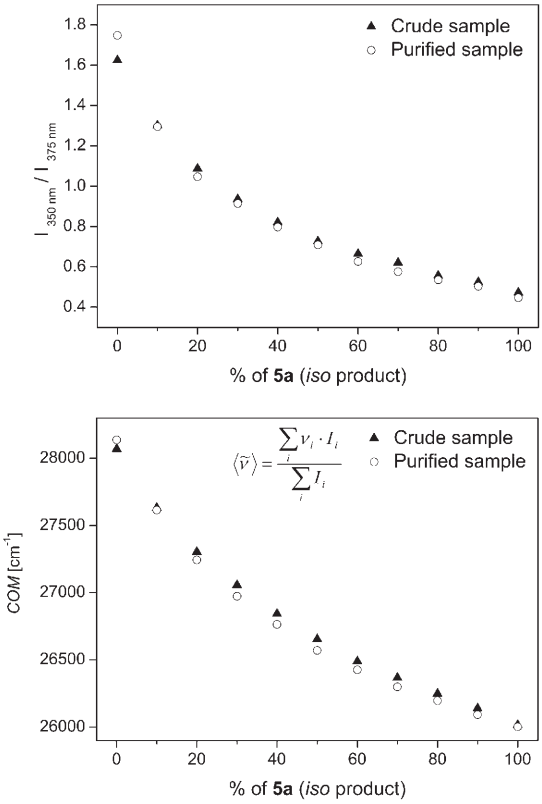


Figure 2. Regioselectivity profiles of both crude and pure **5a** and **6a** and their mixtures in DMSO, λ_{ex} = 275 nm, c = 20 μM at 25 °C.

Dual calculations were performed for the determination of the isomeric ratio. First, the dependence of the ratio of the fluorescence intensities at the emission maxima of the respective pure isomers on the isomeric ratio was calculated. For comparison, the dependence of the center of spectral mass (*COM*, $\langle\tilde{\nu}\rangle$) on the isomeric ratio was also calculated. Both approaches are expected to be rather insensitive to the particular instrumental set-up and are independent of the fluorophore concentration, as long as inner filter effects or self-quenching due to complex formation can be ruled out. Hence, data from different spectrometers are likely to be comparable.

To further determine the scope and limitations of this assay, other novel substrates that bore substitution on either the styrene (**5b** and **6b**) or the phenylhydrazine (**5c** and **6c**) were run through the tandem reaction and their fluorescence spectra were measured. When a *p*-chlorostyrene was employed in the reaction, an even greater change (*ca.* 60 nm) in the maxima of the fluorescence spectra than was present in the initial pair was noticed (Figure 3). As expected, similar results were noticed on both standard curves for the determination of the isomeric ratio (Figure 4).

An additional test of the breadth of this assay came from the use of indoles **5c** and **6c**, prepared from styrene and *p*-chlorophenylhydrazine (Figure 5). These compounds exhibit different spectral properties than the previous pairs in that they have no noticeable difference in fluorescence maxima. Nevertheless, they show great changes in their fluorescence intensity (**6c** is approximately 60-fold stronger than **5c**). Taking advantage of this fact, the intensities of **5c** and **6c** and their mixtures can be plotted directly, easily allowing for the determination of the ratio of regioisomers present (Figure 6).

As should be expected, all three pairs of indoles – those lacking any substitutents or those bearing chlorine as a substituent anywhere on the indole or arene system – exhibited different spectral characteristics. In spite of these differences, we were able in all cases to equate the spectra and calculate the dependence of fluorescence intensities at the emission maxima with the isomeric ratio.

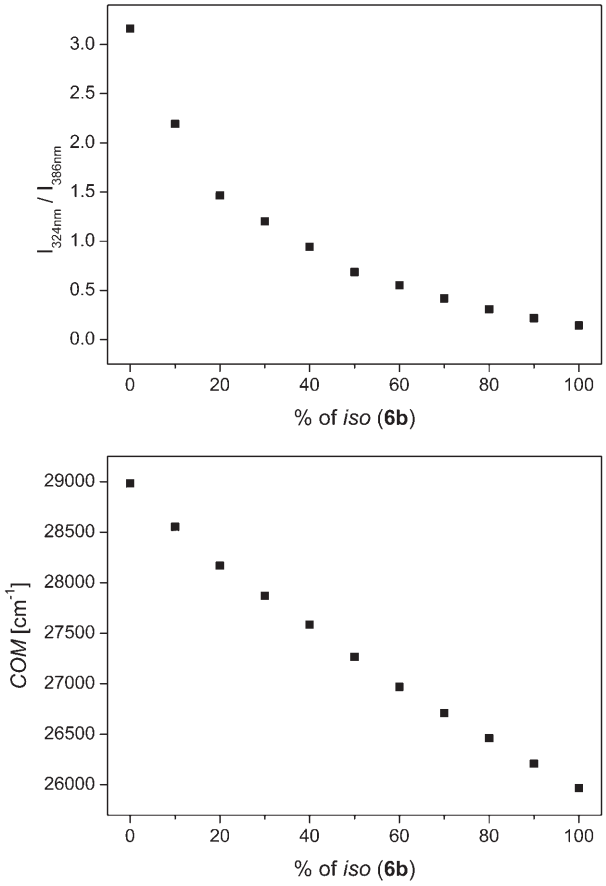


Figure 4. Ratio of the fluorescence intensity at the two emission maxima (of *n*- and *iso*-regioisomer, respectively) (*top*) and *COM* of the emission spectra (*bottom*) *vs.* the molar ratio of the two regioisomers of **5b**/**6b** pair at $\lambda_{ex}=275$ nm.

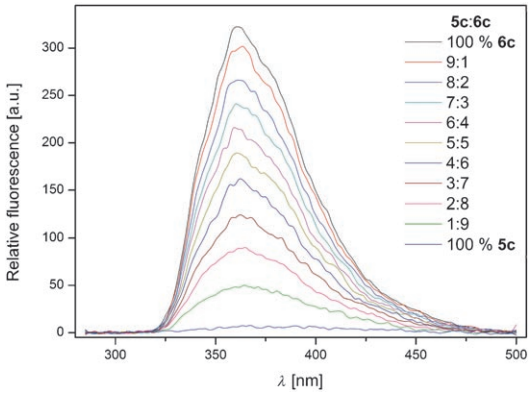


Figure 5. Fluorescence spectra of **5c**, **6c** and their mixtures ($\lambda_{ex}=275$ nm, DMSO, $c=20$ μM, 25 °C).

With the rapidity and tolerance of this assay demonstrated, comparing its reliability and accuracy to the standard technique of ^{1}H NMR spectroscopy was performed as a final test. A mixture of aldehyde isomers (obtained from the hydroformylation of styrene or by mixing known amounts of the corresponding commercial products), was trapped with phenylhydrazine and the corresponding pair of indoles is obtained. The ratio of the products was determined by obtaining the fluorescence spectra and calculating the ratio from the standard curves. Finally, the result obtained from the fluorescence assay was compared with the ratio determined *via* ^{1}H NMR spectroscopy. The results indicate that further investigation and development of this method is most certainly warranted. Over 65 % of the trials deviated by less than ±5 % from the result shown by NMR. Less than one run in ten deviated by more than ±10 % from the integrated NMR spectra.

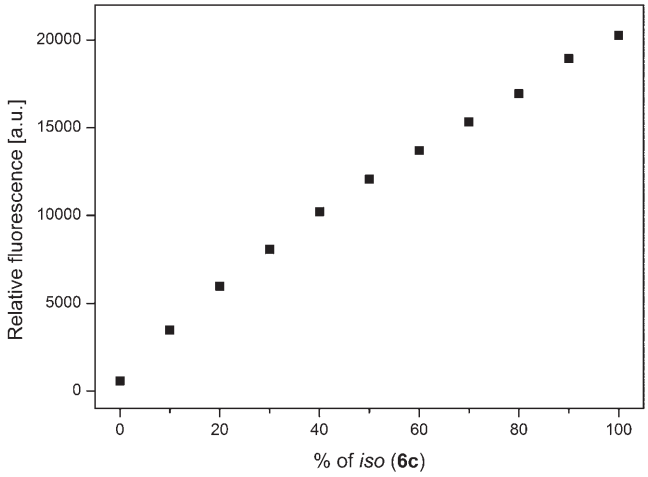


Figure 6. Maximum emission intensity ($\lambda_{em}=361$ nm) *vs.* ratio of the regioisomers ($\lambda_{ex}=275$ nm) of the **5c**/**6c** pair.

Conclusions

In conclusion, we have reported a rapid and reliable fluorescence-based assay for the measurement of regioselectivity. Notably, the assay allows for the analysis of crude reaction mixtures and has proven itself superior to gas chromatography in the handling of unstable reaction products. In addition, the accuracy of the assay is comparable to that of NMR spectroscopy. This method brings together two seldom-paired disciplines of chemical science and opens a new realm of applications of fluorescence spectroscopy as an analytical tool in organic synthesis. We demonstrated its use in the determination of regioisomers produced in the tandem hydroformylation/Fischer indole synthesis, and reliable results were obtained in all cases. As fluorescence is the sole requirement for its employment, this method allows for the creation of specific assays for other bond-forming reactions, target compounds or isomers.

Experimental Section

General Remarks

All chemicals were purchased from commercial sources and were used without further purification. Solvents were purified using standard procedures.[13] 3-Phenylpropionaldehyde (hydrocinnamaldehyde, **2a**) and 2-phenylpropanal (hydratropaldehyde, **3a**) were purchased from Fluka. All reactions with air-sensitive compounds were carried out in dry reaction vessels under an atmosphere of dry argon. ^{1}H and ^{13}C NMR spectra were recorded at room temperature with Bruker DRX 400 and DRX 500 spectrometers. The 1D-NOESY experiment was performed on a Varian Inova 600. If not otherwise specified, $CDCl_3$ was used as solvent with TMS as an internal standard. ^{13}C NMR spectra were referenced to $CDCl_3$ (77.00 ppm). Mass spectra (FAB) were recorded using a JEOL JMS-SX 102 A spectrometer. FT-IR spectra were recorded as thin films on salt plates or as KBr pellets. The peak intensities are defined as very strong (vs), strong (s), middle (m) or weak (w). Fluorescence measurements were performed with a Perkin–Elmer LS 50B spectrofluorometer at 25 °C. Column chromatography was performed using silica gel 60 (70–230 mesh ASTM) from Macherey–Nagel GmbH & Co. KG, Düren, with a mixture of cyclohexane and MTBE or EtOAc as eluent. Pressure reactions were carried out in autoclaves of type A (250 mL, PTFE insert) from Berghof, Eningen or similar house-made autoclaves (100 mL, stainless steel) with specially designed heating and stirring mantles.

General Procedure for Reactions in the Autoclave

After charging the autoclave with the starting material, the catalyst precursor and the solvent, the reactor was flushed with argon, pressurized with gases (carbon monoxide and hydrogen), and heated to the required reaction temperature. Following the reaction, the solvent was removed by rotary evaporation and the products were purified by column chromatography.

General method A (indoles directly from olefins): 1 equiv. olefin, 1 equiv. arylhydrazine, 1 equiv. *p*-toluenesulfonic acid and $Rh(acac)(CO)_2$ (0.5 mol %) were diluted in anhydrous THF, filled in an autoclave and pressurized with CO and H_2. After stirring at 100 °C for 2 or 3 days, the mixture was washed with aqueous ammonia and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel.

General method B (indoles *via* hydrazones): 1 equiv. olefin, 1 equiv. arylhydrazine and $Rh(acac)(CO)_2$ (0.5 mol %) were diluted in anhydrous THF, filled in an autoclave and pressurized with CO and H_2. After stirring for 3 d at 100 °C, the solvent was evaporated. The crude hydrazone was dissolved in 4 % H_2SO_4/THF (w/w). The reaction mixture was refluxed for 3 h, cooled to the room temperature, washed with aqueous ammonia and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel.

General method C (indoles from aldehydes): 1 equiv. of the pure or mixed **2a** and **3a** (*n*- and *iso*-aldehydes) and 1 equiv. of **4a** were diluted in 4 % H_2SO_4/THF (w/w). The reaction mixture was refluxed for 3 h, cooled to the room temperature, washed with aqueous ammonia and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel.

Preparation of 3-Benzyl-1*H*-indole (5a) and 3-Methyl-2-phenyl-1*H*-indole (6a)

Method 1: Following general procedure A, 0.32 g (3.1 mmol) of **1a** and 0.32 g (3.0 mmol) of **4a** were stirred in anhydrous THF for 2 d. After purification 0.05 g (8 %) of **5a** were isolated along with 0.19 g (31 %) of **6a**. The spectroscopic data matched that reported in the literature.[14]

Method 2: Following general procedure C, **5a** and **6a** were synthesized in quantitative amounts when starting from **2a** or **3a** and phenylhydrazine.

Preparation of 3-(4-Chlorobenzyl)-1*H*-indole (5b) and 2-(4-Chlorophenyl)-3-methyl-1*H*-indole (6b)

Following general procedure A, 0.28 g (2.0 mmol) of **1b** and 0.23 g (2.1 mmol) of **4a** were stirred in anhydrous THF for 2 d. After purification 0.09 g (20 %) of **5b** were isolated along with 0.21 g (48 %) of **6b**, both as yellow solids.

5b: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.00$ (s, 2H, CH_2), 6.83 (s, 1H, C*H*), 6.98–7.41 (8H, 8 × C*H*), 7.90 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 31.0$ (*C*H$_2$), 111.1 (*C*H), 115.3 (C_q), 119.0 (*C*H), 119.5 (*C*H), 122.2 (*C*H), 122.3 (*C*H), 126.5 (C_q), 128.4 (2 × *C*H), 130.0 (2 × *C*H), 131.6 (C_q), 139.7 (C_q), 148.0 (C_q); FAB-MS: *m/z* (%) = 242 ($M+H^+$, 49), 241 (M^+, 100), 74 (29); IR: $\tilde{\nu} = 3408$ (m), 3051 (w), 2920 (w), 1491 (s), 1090 (s), 744 (vs) cm^{-1}; FAB-HR-MS: calculated for $C_{15}H_{12}NCl$: 241.0658 g mol^{-1}; found: 241.0661 g mol^{-1}.

6b: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.35$ (s, 3H, CH_3), 7.03–7.17 (2H, 2 × C*H*), 7.25–7.53 (6H, 6 × C*H*), 7.88 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 9.6$ (*C*H$_3$), 109.2 (C_q), 110.7 (*C*H), 119.1 (*C*H), 119.7 (*C*H), 122.6 (*C*H), 128.9 (2 × *C*H), 129.0 (2 × *C*H), 129.9 (C_q), 131.7 (C_q), 132.8 (C_q), 133.2 (C_q), 135.9 (C_q); FAB-MS: *m/z* (%) = 242 ($M+H^+$, 43), 241 (M^+, 100), 77 (89); IR: $\tilde{\nu} = 3419$ (vs), 3050 (w), 2925 (w), 1643 (w), 1493 (w), 1092 (m), 744 (vs) cm^{-1}; FAB-HR-MS: calculated for $C_{15}H_{12}NCl$: 241.0658 g mol^{-1}; found: 241.0670 g mol^{-1}.

Preparation of 1-Chloro-4-iodobenzene

4-Chloroaniline (8.23 g, 88 mmol) was suspended in 12 mL H_2SO_4 and 100 mL water and cooled to 0 °C. $NaNO_2$ (6.10 g, 88 mmol) dissolved in 20 mL water was added. The reaction mixture was filtered into a solution consisting of 22.53 g (150 mmol) NaI and 75 mL water. After stirring at room temperature for 15 min the mixture is extracted with MTBE, dried over $MgSO_4$ and the solvent is removed to afford 1-chloro-4-iodobenzene; yield: 12.70 g (60 %).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.08$ (d, $^3J = 8.7$ Hz, 2H, 2 × CH_{ar}), 7.61 (d, $^3J = 8.7$ Hz, 2H, 2 × CH_{ar}); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 109.6$ (C_q), 130.5 (2 × CH_{ar}), 133.0 (C_q), 138.7 (2 × CH_{ar}). The spectroscopic data matched those reported in the literature.[15]

Preparation of *N*-(4-*C*hlorophenylhydrazine)-carboxylic Acid *tert*-Butyl Ester (4b)

1-Chloro-4-iodobenzene (2.98 g, 13 mmol), $BocNHNH_2$ (1.98 g, 15 mmol), cesium carbonate (5.70 g, 18 mmol), CuI (0.12 g, 1 mmol) and phenanthroline (0.23 g, 1 mmol) were dissolved in 13 mL DMF and heated to 80 °C for 3 d. The reaction mixture was filtered through silica with ethyl acetate, washed with water and dried over $MgSO_4$. After column chromatography **4b** was isolated; yield: 1.60 g (56%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.50$ (s, 9H, 3 × CH_3), 4.39 (bs, 2H, NH_2), 7.25 (d, $^3J = 8.7$ Hz, 2H, 2 × CH_{ar}), 7.42 (d, $^3J = 8.7$ Hz , 2H, 2 × CH_{ar}); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 28.3$ (3 × CH_3), 82.2 (C_q), 124.4 (2 × CH_{ar}), 128.2 (2 × CH_{ar}), 129.7 (C_q), 142.5 (C_q), 153.0 (C_q); MS (FAB): *m/z* (%) = 243 ($M+H^+$, 24), 242 (M^+, 22), 187 (100), 142 (45), 58 (79); IR (KBr/Film): $\tilde{\nu} = 3310$ (m), 3010 (w), 2982 (m), 1696 (vs), 1491 (s), 1335 (s), 1160 (m) cm^{-1}; HR-MS (FAB): calculated for $C_{11}H_{15}N_2O_2ClNa$: 265.0720 g mol^{-1}; found: 265.0723 g mol^{-1}.

Preparation of 3-Benzyl-5-chloro-1*H*-indole (5c) and 5-Chloro-3-methyl-2-phenyl-1*H*-indole (6c)

Following general procedure B, 0.22 g (2.1 mmol) of **1a** and 0.49 g (2.0 mmol) of **4b**[16] were stirred in anhydrous THF for 3 d. After purification 0.13 g (26%) of **5c** were isolated along with 0.21 g (44%) of **6c**, both as yellow solids.

5c: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.07$ (s, 2H, CH_2), 6.95 (s, 1H, C*H*), 6.98 (d, $J = 8.7$ Hz, 1H, C*H*), 7.13 (d, $J = 8.7$ Hz, 1H, C*H*), 7.16–7.22 (2H, 2 × C*H*), 7.25–7.31 (3H, 3 × C*H*), 7.48 (s, 1H, C*H*), 7.90 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 31.4$ (CH_2), 112.1 (*C*H), 115.6 (C_q), 118.6 (*C*H), 120.5 (C_q), 122.3 (*C*H), 122.7 (*C*H), 125.0 (C_q), 126.0 (*C*H), 128.4 (2 × *C*H), 128.6 (2 × *C*H), 131.8 (C_q), 136.5 (C_q); MS (FAB): *m/z* (%) = 242 ($M+H^+$, 49), 241 (M^+, 100), 91 (63); IR: $\tilde{\nu} = 3428$ (m), 3034 (w), 2929 (w), 1601 (m), 1493 (s), 1265 (vs), 739 (vs) cm^{-1}; HR-MS (FAB): calculated for $C_{15}H_{12}NCl$: 241.0658 g mol^{-1}; found: 241.0677 g mol^{-1}.

6c: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.43$ (s, 3H, CH_3), 7.16 (d, $J = 8.4$ Hz, 1H, C*H*), 7.28 (d, $J = 8.4$ Hz, 1H, C*H*), 7.39 (dd, $J = 7.2$; 7.2 Hz, 1H, C*H*), 7.50 (dd, $J = 7.2$; 7.5 Hz, 2H, 2 × C*H*), 7.55–7.60 (3H, 3 × C*H*), 8.04 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 9.6$ (CH_3), 108.4 (C_q), 111.6 (*C*H), 118.5 (*C*H), 122.5 (*C*H), 125.2 (C_q), 127.7 (*C*H), 127.8 (2 × *C*H), 128.9 (2 × *C*H), 131.2 (C_q), 132.8 (C_q), 134.1 (C_q), 135.5 (C_q); MS (FAB): *m/z* (%) = 242 ($M+H^+$, 63), 241 (M^+, 100); IR: $\tilde{\nu} = 3399$ (m), 3067 (w), 2924 (w), 1601 (m), 1495 (s), 1090 (m), 734 (vs) cm^{-1}; HR-MS (FAB): calculated for $C_{15}H_{12}NCl$: 241.0658 g mol^{-1}; found: 241.0661 g mol^{-1}. The structure was clarified by 1D-NOESY experiments.

Determination of the Regioisomeric Ratio

Stock solutions of pure *n*- (**5a**) and *iso*- (**6a**) products were prepared and diluted to the concentration of 20 μM. Nine mixtures with the same concentration of this pair were prepared (*n*:*iso* = 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1). Fluorescence spectra of every mixture were recorded, and the dependency of the ratio of emission intensities and the center of spectral mass (*COM*) of the ratio of isomers were calculated, respectively. The standard curves thus obtained were each fitted with a double-exponential function:[17]

$$y = y_0 + y_1 \cdot e^{-x/t_1} + y_2 \cdot e^{-x/t_2} \quad (1)$$

where y = measured fluorescence signal (ratio of intensities at the emission maxima of the pure isomers/center of spectral mass/emission intensity at a fixed wavelength, respectively), x = percentage of *iso*-regioisomer, y_0 = signal for pure *n*-isomer, y_1, y_2 = amplitudes of the double-exponential function, and t_1, t_2 = corresponding exponential factors.

For the purpose of the fluorescence experiment, samples with "unknown" ratios were prepared with the same concentration (20 μM in DMSO) as with pure products. Upon recording of the fluorescence spectra and calculating relevant values for the $In_{max}/Iiso_{max}$ and *COM*, the *n*:*iso* ratio was calculated according to exponential decay fit [Eq. (1)] and values were compared with ones calculated from ^{1}H NMR (see Table 1 in the Supporting Information).

Samples with "unknown" ratios were synthesized *via* general procedure B or C:

***Via* general procedure B:** After the hydroformylation of styrene (**1a**) and trapping of aldehydes with phenylhydrazine, the mixture of regioisomeric indoles **5a** and **6a** was obtained in its crude form. The ratio of isomers was determined *via* ^{1}H NMR and compared with one obtained after the fluorescence experiment.

***Via* general procedure C:** The arbitrary mixture of commercial aldehydes **2a** and **3a** was trapped with phenylhydrazone into the mixture of regioisomeric indoles **5a** and **6a**. The obtained indole ratio was the same as that of the starting aldehydes (determined *via* ^{1}H NMR of the crude indole mixture), and was compared with the ratio obtained after the fluorescence experiment.

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[16] The Boc-protected hydrazine is used instead of an unprotected one since the compound is more stable against oxidation due to the protection of the nitrogen.

[17] A double-exponential function was arbitrarily chosen for curve fitting since this approach gave satisfactory fits with high correlation coefficients ($R^2 > 0.99$).

