Heterocycles

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Catalytic Asymmetric Synthesis of Mixed 3,3′-Bisindoles and Their Evaluation as Wnt Signaling Inhibitors**

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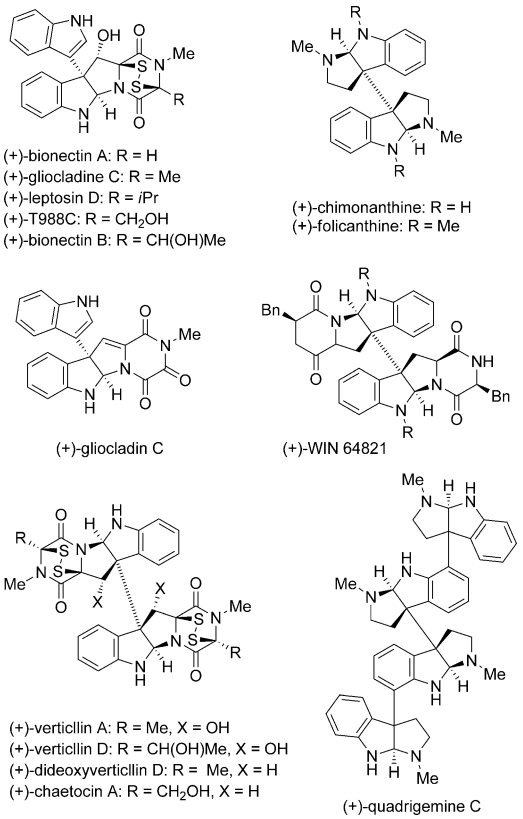
Indole alkaloids possess significant biological activity, and are important compounds in pharmaceutical science.[1] Among the large number of indoles, molecules in which two indoles are connected at the C3- and C3′-positions represent a unique family of bisindoles.[2] In addition to the staurosporines[3] and bisindolylmethanes,[4] directly connected bisindoles at the C3- and C3′-positions are also included in this category (Scheme 1).[5]

In the total synthesis of hexahydropyrroloindoline alkaloids, the construction of dimeric 3,3′-bisindoles has been achieved by the radical coupling of two chiral indoles.[6] Kanai, Matsunaga, and co-workers reported the total syntheses of chimonantine and folicanthine by a catalytic asymmetric double Michael reaction of bisoxindole, which was prepared by condensation of oxindole and isatin.[7] However, in the development of more diverse unsymmetrical bisindoles, chemoselective transformation after the construction of the bisindole skeletons is disadvantageous because the reactivity of two indole moieties in a single molecule is difficult to control. The research groups of Overman[8] and Trost[9] reported the stereoselective total synthesis of (+)-gliocladin C from racemic, unsymmetrical 3-indol-3′-yloxindoles, synthesized by a Rh-catalyzed coupling reaction of indoles with an isatin-derived diazo compound. Stephenson and co-workers developed an interesting visible-light photoredox catalytic unsymmetrical coupling of pyrroloindolines with indoles for the total synthesis of (+)-gliocladin C.[10]

These pioneering works suggest that the coupling reaction of two indole substrates having different oxidation states could be an alternative and more concise approach for unsymmetrical bisindoles, even though successful examples of the catalytic asymmetric synthesis of chiral unsymmetrical 3,3′-bisindoles are limited. Wang, Li, and co-workers reported the organocatalytic synthesis of 3-indolyl-3-hydroxy-2-oxindoles by an enantioselective Friedel–Crafts reaction of indoles with isatins.[11] Guo, Peng, and co-workers constructed a chiral quaternary carbon center from racemic 3-indolyl-3-hydroxy-2-oxindoles in an asymmetric α-alkylation of ketones.[12]



Scheme 1. Examples of chiral bisindoles directly connected at C3- and C3′-positions (3,3′-bisindole).

Herein, we describe the first examples of a catalytic asymmetric coupling reaction of indoles with isatin-derived nitroalkenes in a catalytic asymmetric 1,4-addition manner, and the evaluation of the biological activities of the newly formed chiral 3,3′-bisindoles by a Wnt signaling inhibitory assay.

For the construction of mixed 3,3′-bisindoles, investigation into the appropriate Lewis acid for promoting the coupling reaction of indole (**1a**) with isatin-derived nitroalkenes (**2a**)

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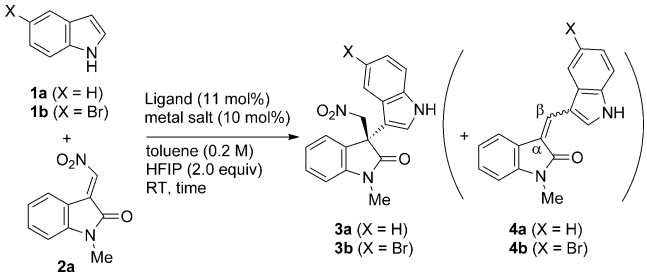
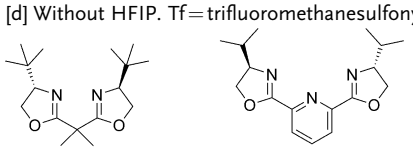
Table 1: Optimization of catalytic asymmetric Friedel–Crafts reaction of indoles with isatin-derived nitroalkenes.[a]

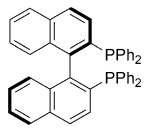


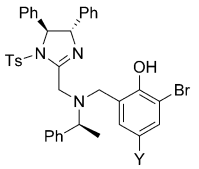
Entry	X	Ligand	Metal salt	*t* [h][c]	Yield of **3** [%]	*ee* of **3** [%]
1	H	–	$Ni(OTf)_2$	40–48	trace	–
2	H	–	$Cu(OTf)_2$	40–48	trace	–
3	H	–	CuOTf	40–48	<41 (<13)[b]	–
4	H	–	$AlCl_3$	40–48	trace	–
5	H	–	BF_3-Et_2O	40–48	<19 (<12)[b]	–
6	H	–	$Yb(OTf)_3$	40–48	21	–
7	H	**L1**	CuOTf	47	<35	*rac*
8	H	**L2**	CuOTf	70	<15	*rac*
9	H	**L3**	CuOTf	70	<16	*rac*
10	H	**IAP1**	CuOTf	13	90	92
11	H	**IAP2**	CuOTf	15	>99	91
12[c]	H	**IAP2**	CuOTf	21	>99	97
13	H	**IAP2**	$Cu(OTf)_2$	19	>99	88
14	Br	**IAP2**	CuOTf	24	79	81
15[d]	Br	**IAP2**	CuOTf	144	50	78
16	Br	**IAP2**	$Cu(OTf)_2$	21	92	87

[a] All reactions used 2 equiv of **1** to **2**. [b] Value of parenthesis is the yield of **4**. [c] The reaction was performed using 2 equiv of **2a** to **1a**.
[d] Without HFIP. Tf = trifluoromethanesulfonyl, Ts = *p*-toluenesulfonyl.


(*S*,*S*)-*t*Bu-box (**L1**)

(*R*,*R*)-*i*Pr-pybox (**L2**)


(*R*)-binap (**L3**)


IAP1 Y = Br
IAP2 Y = NO_2

was conducted (Table 1). However, the reaction is not as easily promoted by Lewis acidic metal salts as expected. When relatively strong Lewis acids were applied (entries 1–6), the reaction was sluggish, and produced complex mixtures. One undesired product was the nonchiral product **4**. The formation of **4** occurred through elimination of the nitro functionality after conjugate addition of **1a** to **2a**, as an α,β-unsaturated enone. Among the conventional Lewis acids examined, CuOTf gave the desired product in moderate yield (entry 3). Chiral ligands were investigated for the CuOTf-catalyzed reaction, in an effort to develop an asymmetric reaction. The reaction proceeded when bisoxazoline (**L1**), bis(oxazoline)pyridine (**L2**), and binap (**L3**) were used, but **3a** was obtained in racemic form and in only moderate yields (entries 7–9). Therefore, previously developed chiral imidazoline-aminophenol ligands, **IAP1** and **IAP2**, were used for the CuOTf-catalyzed Friedel–Crafts reaction between an indole and a nitroalkene.[13] Under conditions reported previously,[13a] **IAP1**/CuOTf smoothly catalyzed the reaction to produce **3a** as the sole product in 90% yield with 92% *ee* (entry 10). The yield of **3a** was improved by the use of the more Lewis acidic **IAP2**/CuOTf catalyst[13b] to achieve quantitative yield and 91% *ee* (entry 11). Interestingly, when 2 equivalents of **2a** were added to **1a**, the enantiomeric ratio of **3a** was improved to 97% *ee* (entry 12). Although this positive effect on the asymmetric induction is interesting, owing to the synthetic value of **2a**, further optimization was achieved for the reaction of 2 equivalents of **1** with **2**. When the less-nucleophilic 5-bromoindole (**1b**) was used, product **3b** was obtained in moderate yield (79%), even from reaction using 2 equivalents of **1b**, and enantioselectivity was lowered to 81% *ee* (entry 14). The positive effect that 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; 2 equiv) exerted on catalyst activity and enantioselectivity was demonstrated when the reaction was performed in the absence of HFIP (entry 15).[13] To further investigate this asymmetric catalysis, the conditions for reaction of **1b** with **2a** were re-examined.

Among the cationic salts of first-row transition metals examined for **IAP2**, the complex prepared using $Cu(OTf)_2$ showed the highest catalytic activity to provide product **3b** in 92% yield with an enantiomeric excess of 87% *ee* (entry 16). In contrast, the use of the **IAP2**/$Cu(OTf)_2$ catalyst gave **3a** with a lower *ee* value (88% *ee*; entry 13) than that obtained for the **IAP2**/CuOTf catalyzed reaction (97% *ee*, entry 11; see the Supporting Information for details).

The appropriate catalyst, that is, either **IAP2**/CuOTf or **IAP2**/$Cu(OTf)_2$, was selected depending on the substrate reactivity (Scheme 2). Reaction of isatin-derived nitroalkene **2a** with indole nucleophiles (**1**) having electron-donating substituents in the 5-position, yielded products (**3g** and **3j**) with greater than 87% *ee*. The reaction of 7-methyl-indole with **2a** was also smoothly catalyzed by **IAP2**/$Cu(OTf)_2$ to give **3f** in quantitative yield with 88% *ee*, although the use of 2-methyl or 4-methyl indole resulted in low yields with moderate *ee* values (**3h** and **3i**). The protecting groups on the indole nitrogen atom of **2** affected the reaction significantly. Although the *N*-methyl, *N*-benzyl, and *N*-allyl substrates (**2**) were successfully converted into product (**3b–d**), the *N*-acetyl product **3e** was obtained in less than 29% yield. for the reactions of 7-methylindole with 5-substituted isatin-derived nitroalkene **2**, the products were obtained with over 90% *ee* (**3k–o**). The 7,7′-dimethyl product (**3p**) was obtained in 89% yield with 83% *ee*. The absolute configuration of product **3b** was determined by X-ray crystallography (Figure 1). For the production of (*S*)-**3b**, we believe compound **1b** attacks the *Re* face of **2a** in the same manner as observed in the previously studied **IAP2**/CuOTf-catalyzed Friedel–Crafts reaction of indoles with nitroalkenes.[13]

Some chemical transformations of the Friedel–Crafts adduct **3b** were examined to demonstrate diversification of 3,3′-bisindoles (Scheme 3). The reduction of nitro groups and Suzuki–Miyaura coupling proceeded smoothly to give the

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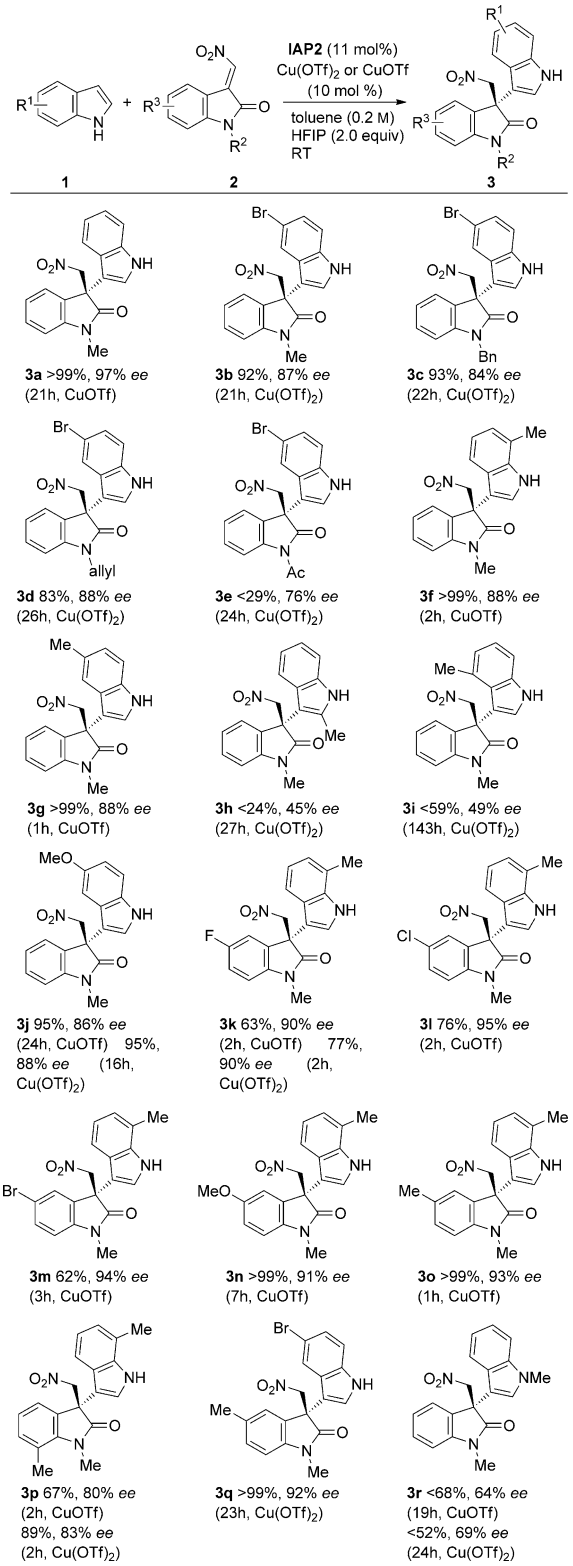


Scheme 2. Details of imidazoline-aminophenol-Cu catalyzed asymmetric Friedel–Crafts reaction of indoles with isatine-derived nitroalkenes.

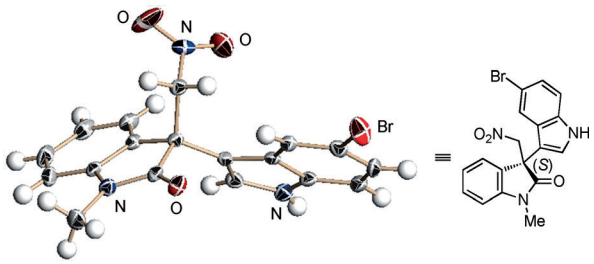
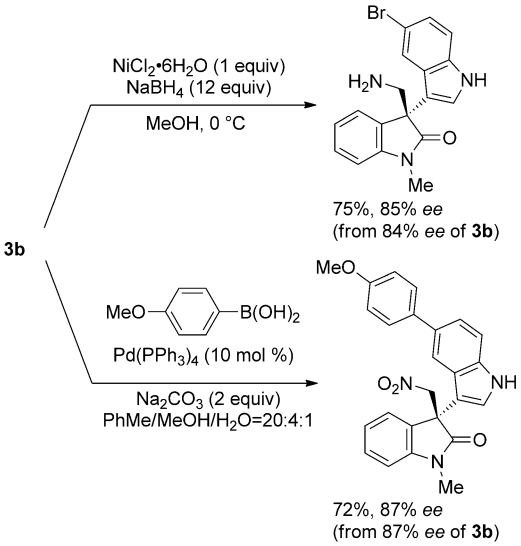


Figure 1. X-ray crystallographic analysis of **3b**.[26]



Scheme 3. Diversification of **3b**.

corresponding compounds without significant loss of enantiomeric excess.

The biological activities of **3** were examined in a Wnt signaling inhibitory assay. The Wnt signaling pathway plays key roles in cell morphology, motility, proliferation, and differentiation. However, mutation or loss of function of signaling component proteins (e.g., APC, Axin) disrupts β-catenin degradation by proteasomes, which causes abnormal accumulation of β-catenin, aberrantly activating this signal. The excess β-catenin translocates to the nucleus where it binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) and stimulates transcription of target genes, including c-Myc and cyclin D, which contribute to tumorgenesis of many cancers, especially colon cancers.[14] Therefore, small molecules that downregulate TCF/β-catenin transcriptional activity would be potential candidates for treating cancer. Potent Wnt signaling inhibitors quercetin,[15] ICG-001,[16] hexachlorophen,[17] IWR-1,[18] and pyrvinium[19] have been recently reported. The bisindole natural product lycogarubin B[20] and a synthetic isomer of the natural product *cis*-dihydroarcyriarubin C[20] also exhibited significant Wnt signaling inhibition.

To investigate the Wnt signaling inhibition of the natural-product-like 3,3′-bisindole products **3** we have used a TOP-Flash assay, which is a cell-based reporter luciferase assay involving TCF/β-catenin transcription in the cell line STF/293[21] with LiCl as a GSK-3β inhibitor for inducing β-catenin accumulation[22] (Figure 2 and Figure S2 in the Supporting Information).[23] Interestingly, many compounds (**3**) showed TCF/β-catenin transcription signaling inhibitory activity (see

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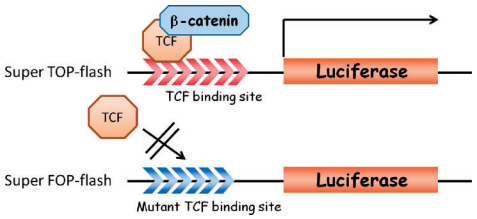

Figure 2. Schematic representation of the assay system involving Super TOP-Flash and Super FOP-Flash reporters.

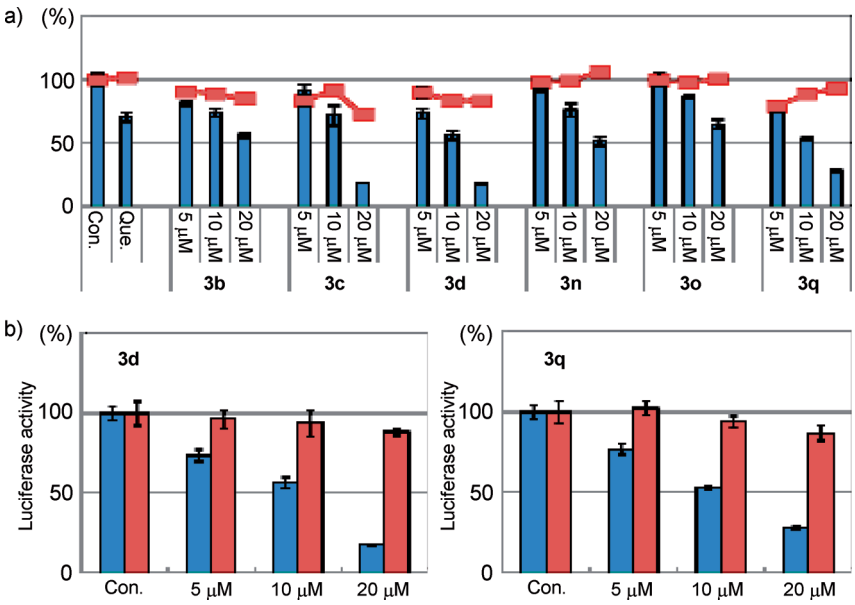

Figure 3. a) Inhibition of TCF/β-catenin transcriptional activity, shown by activation of the Super TOP-Flash reporter (blue bars), and cell viability (red). b) Results of TOP-Flash (blue bars) and FOP-Flash (red bars) assays of **3d** and **3q**. The FOP-Flash assay was carried out as transient luciferase reporter assay by using Super FOP-Flash reporter and control pRL-TK vectors in 293T cells. Quercetin (Que.) was used as a standard positive compound (27.7 μM).[15] The control (DMSO) luciferase activity was normalized to 100%. N = 3. Error bars represent SD.

the Supporting Information was detailed results). Representative examples showing significant inhibition and relatively low toxicity are summarized in Figure 3a (viability marked with red color). For example, **3d** and **3q** exhibited inhibition activity with an IC_{50} of 11.2 μM and 11.0 μM, respectively. Although **3c** also showed strong inhibition activity (IC_{50} = 13.3 μM), some cytotoxicity was observed at 20 μM. Notably, **3c**, **3d**, and **3q** are the products from the reactions of 5-bromoindole (**1b**). For realizing the mode-of-action of **3d** and **3q**, we next examined their potency towards β-catenin/TCF transcriptional inhibition by using a FOP-Flash assay.[24,25] Because the TCF binding site of the FOP-Flash reporter plasmids are mutated, this assay has been widely utilized for determining false-positive results. If the inhibition by compounds **3d** and **3q** is caused by other Wnt signal events, such as normal protein expression or proteasome activity, Super FOP-Flash luciferase activity (Figure 3b; red bars) would be also decreased. Compounds **3d** and **3q**, which were shown to by potent inhibitors by the TOP-Flash experiments, did not have any affect on Super FOP-Flash activity, thus indicating these compounds would be the potent Wnt signal inhibitors.

In conclusion, the first efficient catalytic asymmetric coupling reaction of indoles with isatin-derived nitroalkenes was accomplished using the chiral imidazoline-aminophenol ligand **IAP2**/$Cu(OTf)_2$ complex. Biological activity of the newly formed chiral 3,3′-bisindoles was also demonstrated in a Wnt signaling inhibitory assay. A detailed evaluation of the biological activity of the diversified chiral 3,3′-bisindoles is now in progress.

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[25] For the use of FOP-Flash assay as the 2nd screening of the potent Wnt signaling inhibitors, see Figure 4 in Ref. [14b].
[26] CCDC 909626 (**3b**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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