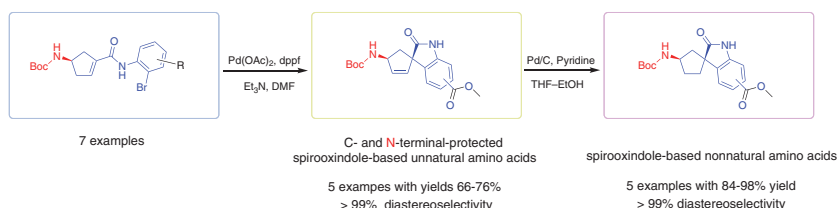


# A Diastereoselective Mizoroki–Heck Reaction for Synthesis of Spirooxindole-Based Nonnatural Amino Acids Using a Boc-Protected Amine Chiral Auxiliary

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**Abstract** Nonnatural amino acids are pivotal for expanding the functional diversity of peptides and proteins, enabling novel therapeutic opportunities. Mono-Boc-protected spirocyclization precursors have been developed as versatile intermediates for the diastereoselective synthesis of spirooxindole-based nonnatural amino acids by the Mizoroki–Heck reaction. Catalytic hydrogenation produced cyclopentyl derivatives, expanding the diversity of these amino acids. Furthermore, one spirooxindole derivative was incorporated into a tripeptide by solid-phase peptide synthesis on Rink amide resin to demonstrate its potential in peptide modification and drug development.

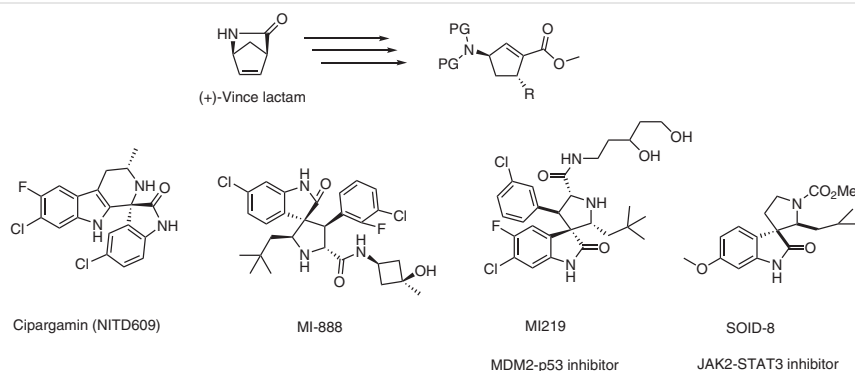
**Key words** amino acids, diastereoselectivity, asymmetric synthesis, Mizoroki–Heck reaction, spirooxindoles, palladium catalysis

The Mizoroki–Heck reaction is a fundamental method for carbon–carbon (C–C) bond formation, and is highly valued for its ability to construct complex molecular structures with precision and efficiency.<sup>1–4</sup> Its utility in creating biologically active molecules, natural products, and stereochemically defined frameworks highlights its indispensability in medicinal chemistry and synthetic innovation. The intramolecular Mizoroki–Heck reaction enables efficient cyclization of alkenes and aryl/vinyl halides, to form stereodefined five- or six-membered rings.<sup>1,2</sup> Key stereose-

lectivity is controlled by ligands, substrate design, reaction conditions, and chiral catalysts. This method is vital for synthesizing complex cyclic molecules, introducing stereocenters, and accessing enantioenriched compounds, with broad applications in pharmaceuticals, natural products, and material science.<sup>3,4</sup>

As part of a medicinal chemistry program aimed at the synthesis of nonnatural  $\gamma$ -amino acids, our group reported a stereoselective functionalization of derivatives of 2-azabicyclo[2.2.1]hept-5-en-3-one (Vince lactam),<sup>5</sup> a common starting material for the synthesis of the carbocyclic nucleoside class of antivirals (Scheme 1). We surmised that the use of a bulky amine protecting group as a chiral auxiliary might control the diastereoselectivity of a Mizoroki–Heck arylation and vinylation of the cyclopentenyl scaffold, producing nonnatural  $\gamma$ -amino acids with protecting groups on both the C- and N-terminals. Spirooxindoles are prominent structural motifs found in a wide range of natural products and synthetic compounds with significant biological activities.<sup>6,7</sup> Additionally, their role in asymmetric synthesis and chiral catalyst design highlights their broad utility in advancing chemical and medicinal research.<sup>8</sup>

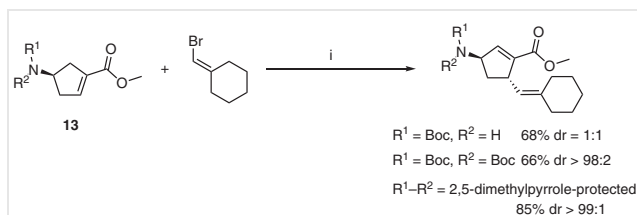
Solid-phase peptide synthesis (SPPS) is a widely used technique for synthesizing peptides both on a laboratory scale and in industrial applications.<sup>9,10</sup> It involves anchoring an N-protected amino acid to a solid phase, sequentially coupling additional amino acids using amide reagents, and cleaving the resulting peptide from the resin. Because the deprotection of 2,5-dimethylpyrrole-protected amines previously used as chiral auxiliaries<sup>11</sup> requires conditions that are incompatible with SPPS, replacing this protecting group



**Scheme 1** Diastereoselective synthesis of Vince lactam-derived cyclopentenyl  $\gamma$ -amino acids, and some potent biologically active spirooxindole derivatives (Wetzel et al.)<sup>8</sup> PG: protecting group.

with an alternative that could be deprotected under the typically milder conditions employed in SPPS was investigated. In a previously reported study in which we examined the directing effect of the 2,5-dimethylpyrrole moiety on the intermolecular Mizoroki–Heck reaction,<sup>12</sup> we also assessed the ability of mono- and di-Boc-protected amines to influence the diastereoselectivity of the reaction.<sup>13</sup> Whereas the reaction between a vinyl bromide and mono-Boc-protected derivatives gave 1:1 mixtures of diastereomers, the di-Boc-protected derivatives provided >98:2 diastereoselectivity, albeit with lower isolated product yields than the corresponding 2,5-dimethylpyrrole-protected substrates (Scheme 2).

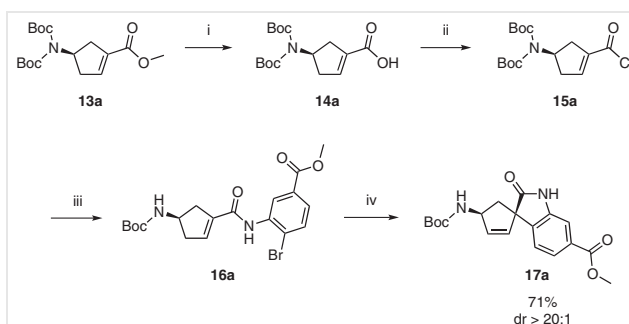
As the preparation of Boc-protected amines is relatively easy and the intermolecular Mizoroki–Heck reaction shown in Scheme 2 provided the product, a method for the intramolecular Mizoroki–Heck synthesis of spirooxindoles bearing Boc protecting groups on the cyclopentenyl amine was investigated. Here, we report a highly stereoselective method for producing spirooxindole-based nonnatural amino acids primed for SPPS applications.



**Scheme 2** The influence of mono-Boc, di-Boc, and 2,5-dimethylpyrrole protection of a chiral amine on the diastereoselectivity of a Mizoroki–Heck reaction. Reagents and conditions: (i)  $\text{Pd}(\text{OAc})_2$  (10 mol%),  $\text{NaHCO}_3$  (2.5 equiv), TBACl (1.1 equiv), DMF, 90 °C, 36 h.

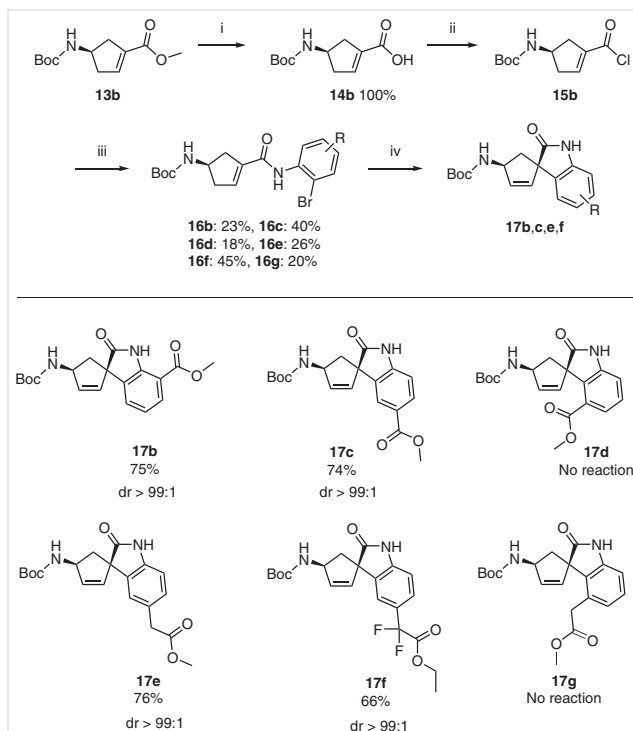
The di-Boc-protected substrate **13a** was synthesized as we previously described.<sup>8</sup> Ester hydrolysis of acrylate **13a** using LiOH in a mixture of THF and water produced **14a**,

which was directly converted into the corresponding acid chloride **15a** using thionyl chloride with a catalytic amount of DMF (Scheme 3). The crude product was treated with methyl 3-amino-4-bromobenzoate that had been preactivated with sodium hydride. The di-Boc-protected amine displayed a poor stability under these conditions, with the mono-Boc-protected **16a** being the major product rather than the desired di-Boc derivative, which was not isolated. The mono-Boc substrate **16a** was subjected to the Mizoroki–Heck spirocyclization protocol for the synthesis of spirooxindoles previously described by Roy et al.,<sup>14</sup> producing **17a** as a single diastereomer in 71% isolated yield. This directing ability of the mono-Boc-protected amine in the cyclization was surprising, as di-Boc-protected amines were required to achieve diastereoselectivity in the intermolecular Mizoroki–Heck application shown in Scheme 2. The difference might be a consequence of increased steric interactions in the intramolecular reaction.



**Scheme 3** Synthesis of the spirooxindole-based nonnatural amino acid **17a** with protecting groups on both the N- and C-terminals. Reagents and conditions: (i) LiOH (5 equiv), 3:1 THF–H<sub>2</sub>O, 50 °C, overnight. (ii)  $\text{SOCl}_2$  (1.2 equiv), pyridine (1.1 equiv), DMF (cat.),  $\text{CH}_2\text{Cl}_2$ , 50 °C, 3 h. (iii) methyl 3-amino-4-bromobenzoate (1.5 equiv), NaH (2 equiv), THF, 50 °C, overnight, 54% (two steps). (iv)  $\text{Pd}(\text{OAc})_2$  (5 mol%), 1,1'-bis(diphenylphosphino)ferrocene (dppf; 10 mol%),  $\text{Et}_3\text{N}$  (2 equiv), DMF, 80 °C, 6 h, 71%.

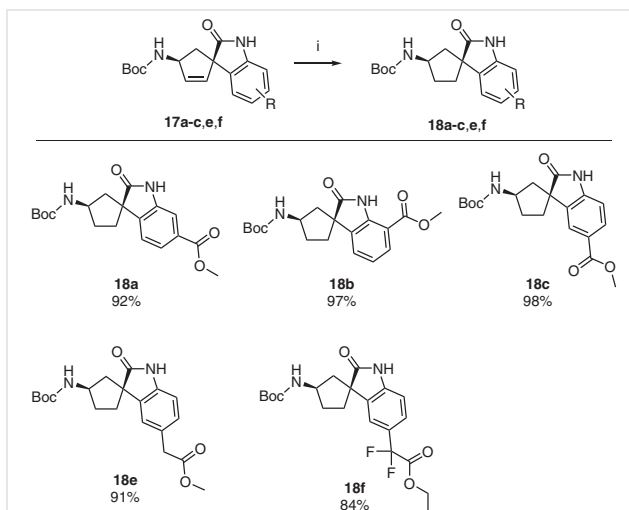
Due to its ability to retain the excellent diastereoselectivity of the spirocyclization reaction with the mono-Boc-protected amine, this synthetic route was adapted to start from the mono-Boc substrate **13b** (Scheme 4). By varying the position of the ester on the aniline, three spirocyclization precursors **16b–f** were prepared in moderate yields. These were subsequently subjected to the Mizoroki–Heck cyclization reaction, producing **17b,c,e,f** in isolated yields of 75, 74, 76, and 66%, respectively. Precursors **16d** and **16g** did not react under these conditions, presumably due to steric crowding at the reactive aryl bromide center.



**Scheme 4** Synthesis of spirooxindoles **17b,c** and **17e,f**. Reagents and conditions: (i) LiOH (5 equiv), 3:1 THF–H<sub>2</sub>O, 50 °C, overnight. (ii) SOCl<sub>2</sub> (1.2 equiv), pyridine (1.1 equiv), DMF (cat.), THF, 50 °C, 3 h. (iii) aniline (1.5 equiv), NaH (2 equiv), THF, 50 °C, overnight, 18–40% (two steps). (iv) Pd(OAc)<sub>2</sub> (5 mol%), dppf (10 mol%), Et<sub>3</sub>N (2 equiv), DMF, 80 °C, 6 h.

The cyclopentenyl products were then reduced by hydrogenation using hydrogen gas (1 atm) with 10 mol% palladium on carbon<sup>8</sup> to give the saturated spirooxindole compounds **18a–c** and **18e,f** in isolated yields of 84–98% (Scheme 5). Moreover, the diastereomeric ratio remained unchanged after the hydrogenation process, confirming the robustness of the stereochemical outcomes under the applied reaction conditions.

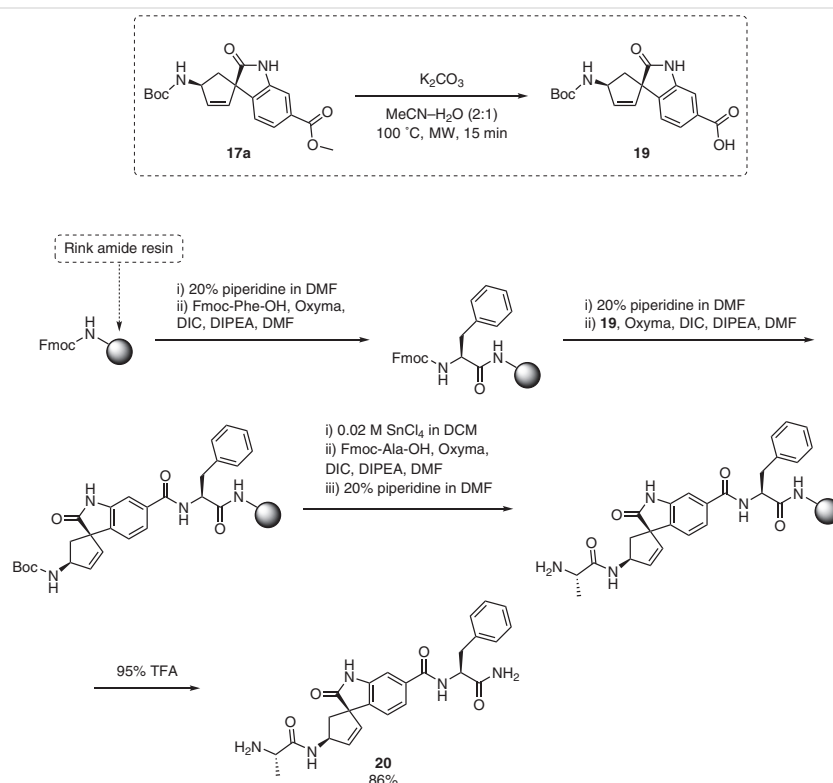
Next, to determine the usefulness of the synthesized spirooxindole-based nonnatural amino acids, attempts were made to include one of these products in a tripeptide by SPPS on Rink amide resin (Scheme 6). The synthesis of the tripeptide **20** was initiated by piperidine-mediated deprotection of the Rink amide Fmoc group, followed by



**Scheme 5** Hydrogenation of **17a–c** and **17e,f**. Reagents and conditions: (i) Pd/C (10 mol%), pyridine (3 equiv), H<sub>2</sub> (1 atm), 1:1 THF–EtOH.

coupling of Fmoc-protected phenylalanine through an Oxy-ma:DIC:DIPEA protocol. The same deprotection-coupling sequence was repeated for the coupling of **19**, formed through ester hydrolysis of **17a** with sodium carbonate in a mixture of acetonitrile and water at 100 °C under microwave heating.<sup>16</sup> As the Rink amide resin is acid-sensitive, this limits the possibilities of using strong acids for Boc deprotection. Thus, a method employing tin(IV) chloride in dichloromethane was used for the Boc deprotection.<sup>15</sup> Following this step, the final Fmoc-protected alanine was coupled by using the previously described coupling protocol. A small portion of the crude product was cleaved from the resin with TFA and analyzed by LC–UV/MS, where the expected mass of the product was successfully detected. However, a small amount of the spirooxindole–phenylalanine dipeptide was also observed, presumably due to incomplete Boc deprotection by tin(IV) chloride. Cleavage of the product from the resin, followed by precipitation in cold diethyl ether, provided the tripeptide product **20** in 86% yield. This initial experiment demonstrates that Boc-protected spirooxindole-based nonnatural amino acids are indeed compatible with SPPS, providing a viable pathway for incorporating our nonnatural amino acid scaffolds into peptides.

In conclusion, we have successfully prepared mono-Boc-protected spirocyclization precursors that provide a powerful approach for the diastereoselective synthesis of spirooxindole-based nonnatural amino acids, significantly enhancing the chemical diversity of available nonnatural amino acids.<sup>17</sup> The use of the Mizoroki–Heck cyclization, followed by catalytic hydrogenation permits the production of cyclopentenyl derivatives, expanding the repertoire of non-natural rigid amino acids. The incorporation of spirooxindoles into peptides through SPPS, demonstrated through the synthesis of a tripeptide on Rink amide resin, under-



**Scheme 6** Preparation of free acid **19** and synthesis of the spirooxindole-containing tripeptide **20** through SPPS on Rink amide resin

scored the utility of this method in peptide modification and the creation of bioactive compounds of biological interest. This strategy holds great promise for advancing both peptide-based drug discovery and protein engineering.

## Conflict of Interest

The authors declare no conflict of interest.

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## Supporting Information

Supporting information for this article is available online at <https://doi.org/10.1055/a-2564-4920>.

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- (17) **18a-c and 18e,f; General Procedure**  
A 5 mL microwave vial was charged with the appropriate spirooxindole **17** (1 equiv) and Pd/C (10 mol%), and 1:1 THF-EtOH (1:1) was added to produce a 0.1 M solution. Pyridine (3 equiv) was then added and the mixture was stirred under  $H_2$  (1 atm) at r.t. for 24 h.  $H_2O$  (5 mL) was added and the mixture was extracted with EtOAc ( $3 \times 15$  mL). The organic phase was washed with brine (5 mL), dried ( $MgSO_4$ ), filtered, and concentrated under reduced pressure.

**Methyl (1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]-2'-oxo-1',2'-dihydrospiro[cyclopentane-1,3'-indole]-6'-carboxylate (18a)**

Synthesized according to the general procedure and purified by flash chromatography (silica gel, 40% EtOAc–isohexane) as a white solid; yield: 32.0 mg (92%);  $[\alpha]_{\text{D}}^{25} = 34.7$  ( $c = 0.1$ , THF).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 8.62$  (s, 1 H), 7.77 (dd,  $J = 7.8, 1.3$

Hz, 1 H), 7.56 (d,  $J = 1.3$  Hz, 1 H), 7.24 (d,  $J = 7.8$  Hz, 1 H), 5.85 (d,  $J = 8.9$  Hz, 1 H), 4.51 (s, 1 H), 3.91 (s, 3 H), 2.39–2.19 (m, 3 H), 2.12–1.86 (m, 3 H), 1.46 (s, 9 H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ):  $\delta = 184.1, 166.7, 155.6, 141.3, 140.4, 130.1, 125.1, 122.5, 110.5, 79.4, 53.7, 53.1, 52.4, 44.3, 36.8, 34.7, 28.6$ . HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_5$ : 361.1758; found: 361.1755.