Synthesis of *N*-Substituted Indoles via Aqueous Ring-Closing Metathesis

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Abstract

We report herein the synthesis of *N*-substituted indoles resulting from the ring-closing metathesis of indole precursors bearing *N*-terminal alkenes. The aqueous metathesis of the indole precursors gave good yields of *N*-substituted indoles (up to 72 %) with commercial metathesis catalysts and with artificial metalloenzymes based on the biotin-streptavidin technology. Strikingly, the yield of the *N*-acetylindole increases in presence of a second metathesis substrate.

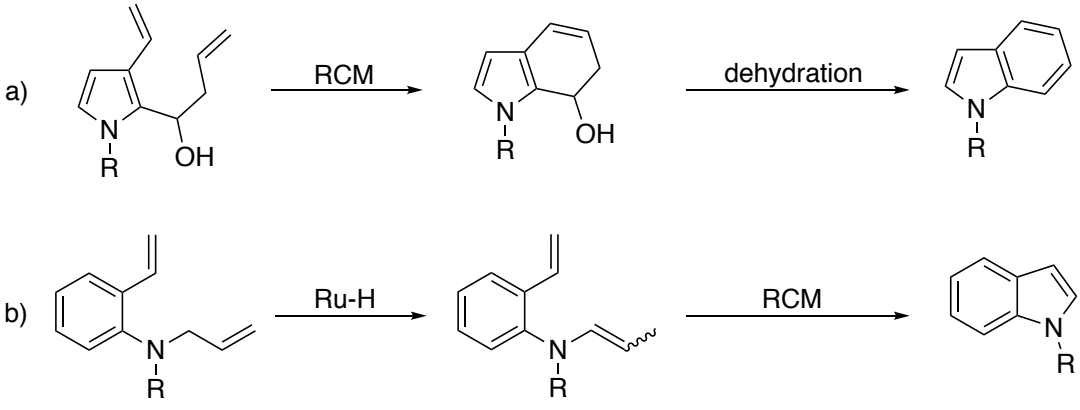
Keywords Aqueous catalysis, Ring-closing metathesis, Homogeneous catalysis, Artificial metalloenzymes

Introduction

Indoles are important synthetic scaffolds [1,2]. The indole core is present in many compounds which possess biological activity, such as naturally-occurring alkaloids and chemotherapeutic drugs [3-6]. Additionally, indole is a metabolite in the biosynthetic pathway of tryptophan, an essential amino acid playing a critical role in the metabolism of eukaryotic and prokaryotic cells [7].

Synthetic strategies relying on ring-closing metathesis (RCM) for the synthesis of indole derivatives include *i*) the formation of a pyrrole ring from a functionalized benzene precursor and *ii*) the formation of a benzene ring from a functionalized pyrrole precursor.

We sought to design suitable substrates for the synthesis of *N*-substituted indoles via aqueous RCM. Two protocols for the RCM of indoles derivatives in organic solvents are displayed in Scheme 1 [8,9]. Yoshida and coworkers generated indoles via a tandem RCM/1,2-elimination [10,11] sequence (Scheme 1a) and Nishida and coworkers reported on a mechanism of selective isomerization of terminal olefins promoted by a ruthenium hydride, followed by RCM to yield indoles (Scheme 1b).



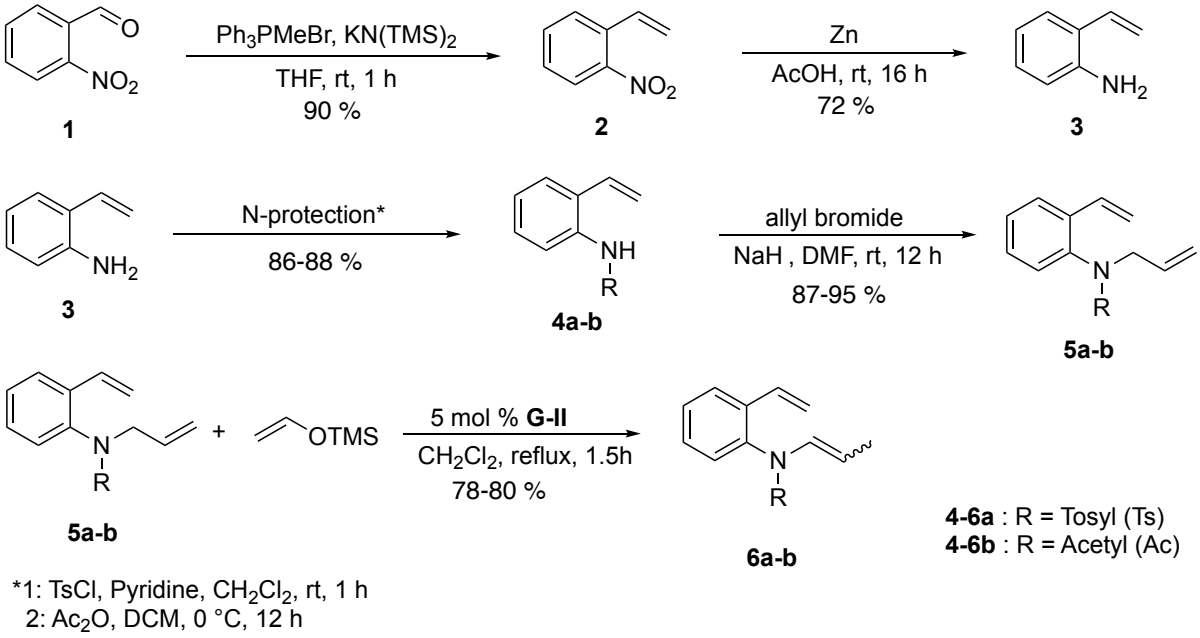
Scheme 1. Reported examples for the synthesis of indole derivatives via ring-closing metathesis [10,11].

Herein, we report on our effort to synthesize *N*-protected indoles starting from *N*-substituted anilides via RCM in aqueous solution. Both homogeneous and an artificial

metathase based on the biotin-streptavidin technology were evaluated, Figure 1 [12-20].

Results and discussion

We initially synthesized the precursors **6a-b** via the synthetic route in Scheme 2. Based on the work of Nishida and coworkers [8], starting form *o*-nitrobenzaldehyde **1**, we reproduced the synthesis of the two substituted indoles **7a-b** by generating the indole precursors **6a-b** containing an internal alkene via a Ru-H promoted isomerization (Scheme 2) [21-24].



Scheme 2. Synthesis of *N*-substituted indole precursors **6a** and **6b**.

Next, we tested the RCM in aqueous buffer under mild conditions with different commercially-available metathesis catalysts (**G-II**, **HG-I**, **HG-II** and **Aquamet**) and with the biotinylated catalyst **Biot-Ru**, Figure 1.

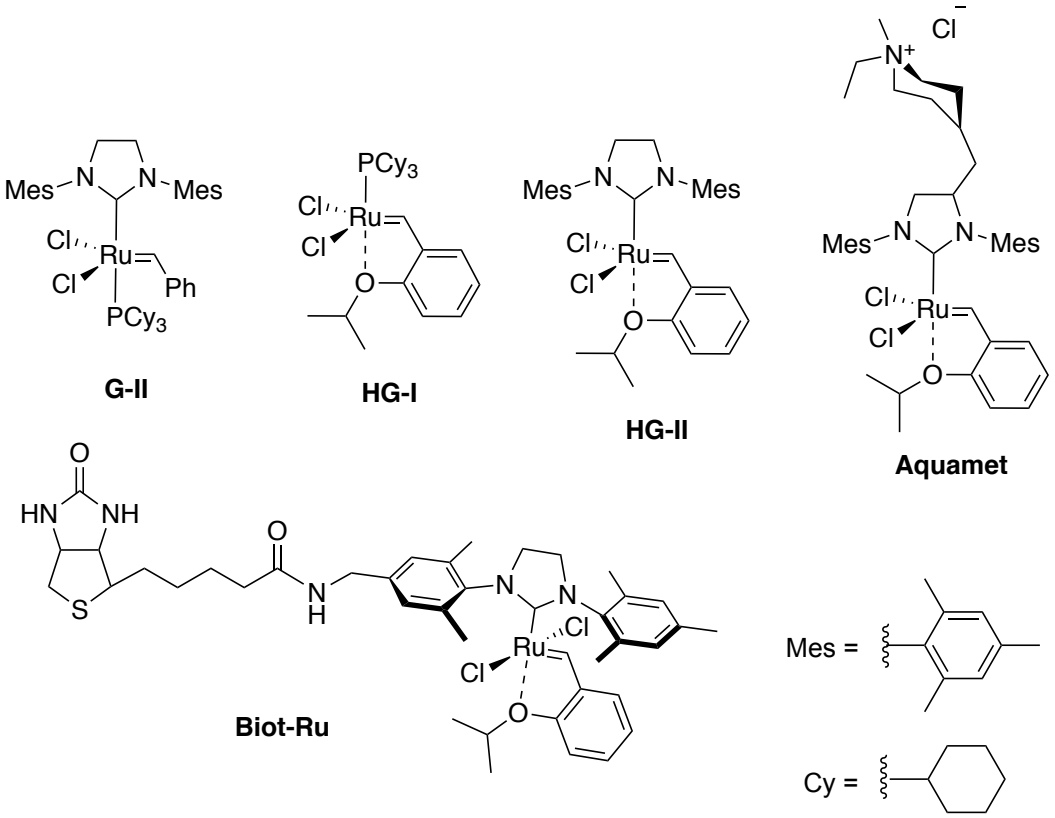
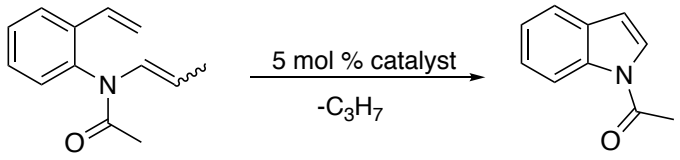


Figure 1. Ruthenium-based metathesis catalysts evaluated for the aqueous RCM of indole precursors.

Substrate **6a** is insoluble in water even in the presence of up to 20 % of organic solvent. It forms either a milky suspension or a precipitate. The solubility in water improves with the *N*-acetylindole precursor **6b**, but activity screening in the presence of different buffers barely achieved a single turnover as summarized in Table 1.

Table 1. RCM of indole precursor **6b** in aqueous buffer.

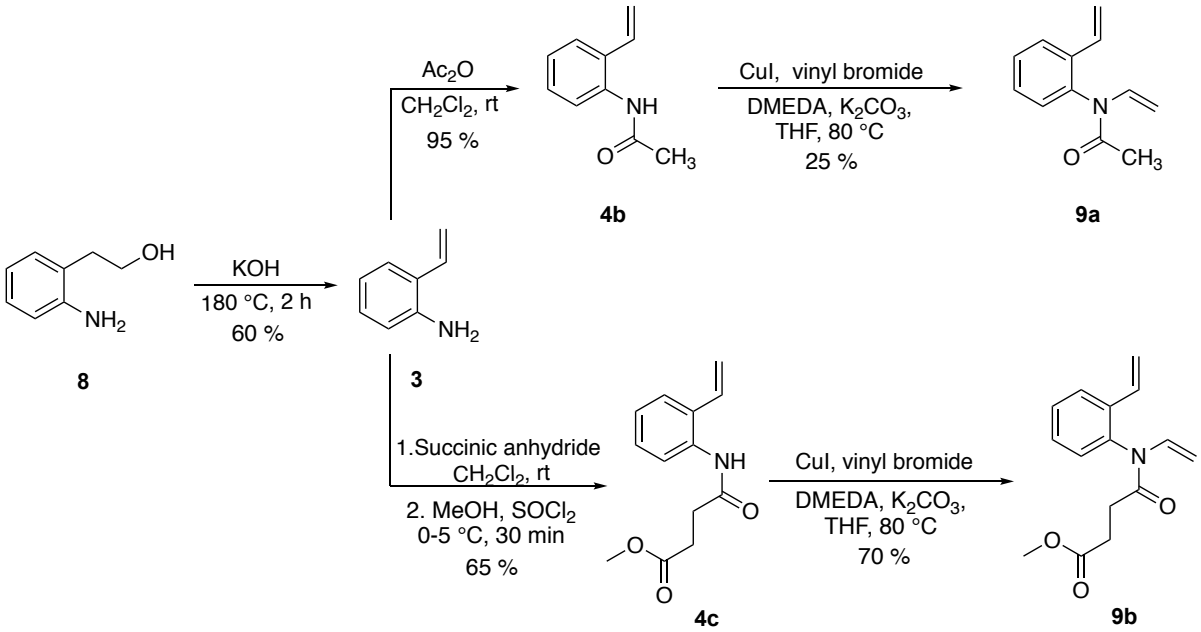


6b (1 mM) **7b**

Entry	Solvent[a]	Catalyst (mol %)	Product (μM)	TON
1	PBS + HSA	**Aquamet** (5)	—	—
2	AcOH/AcONa + HSA	**Aquamet** (5)	—	—
3	PBS	**Aquamet** (5)	—	—
4	AcOH/AcONa	**Aquamet** (5)	60.5	1.2
5	PBS	**Aquamet** (10)	0.0	—
6	PBS	**Aquamet** (10)	0.0	—
7	AcOH/AcONa	**Aquamet** (10)	104.8	1.0
8	AcOH/AcONa	**Aquamet** (10)	71.8	—
9	PBS	**HG-II** (10)	1.4	—
10	AcOH/AcONa	**HG-II** (10)	16.7	—

[a] PBS: phosphate buffer saline, pH 7.4. HSA: human serum albumin, 0.5 mM. Substrate concentration: 1.0 mM. Reactions were carried out at 40 °C for 24 hours.

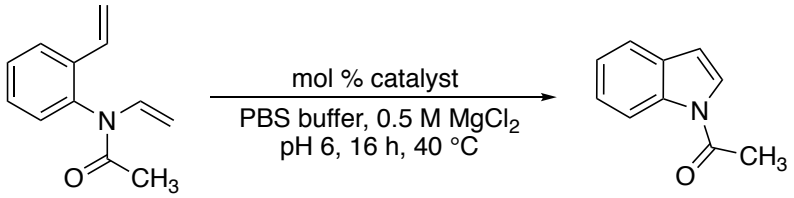
Speculating that RCM with an internal olefin is more challenging in aqueous solution [25], we designed the *N*-vinylanilide derivatives **9a** and **9b**, Scheme 3. These substrates are conveniently synthesized in three steps starting from the neat distillation of the commercially available 2-(2-aminophenyl)ethan-1-ol **8** [26], to afford 2-vinylaniline **3**. The amino group is functionalized with the acetyl or succinyl appendages to yield respectively **4b** and **4c**. The last step is a Cu-catalyzed *N*-vinylation of the secondary amine to afford substrates **9a** and **9b**, Scheme 3.



Scheme 3. Synthesis of the *N*-acetylindole precursor **9a** and *N*-succinylindole methyl ester precursor **9b**.

Table 3 summarizes the results of the aqueous RCM with the substrate **9a**. The RCM activity assay reveals modest to good yields of indole **7b** with four metathesis catalysts, among which **G-II** (10 mol % catalyst loading) gave the highest yield of *N*-acetylindole (72 %, Table 2, entry 6). The water-soluble catalyst **Aquamet** (5 mol % catalyst loading) afforded 66 % of indole **7b** (Table 2, entry 7).

Table 2. Summary of the screening results for the aqueous RCM of substrate **9a**.[a]



9a (0.5 mM) **7b**

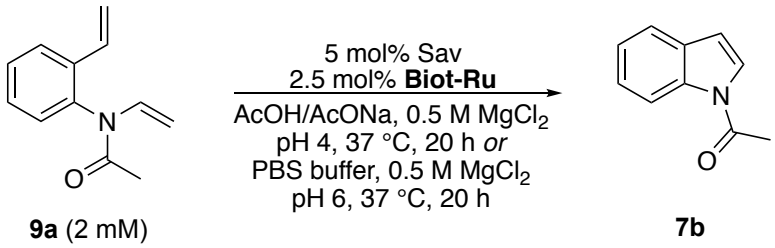
Entry	Catalyst (mol %)	Yield (%)[b]	TON
1	**Biot-Ru** (5)	35 ± 10	7 ± 2
2	**Biot-Ru** (10)	41 ± 15	4 ± 1.5
3	**HG-I** (5)	56 ± 5	11 ± 1
4	**HG-I** (10)	63 ± 0	6 ± 0
5	**G-II** (5)	67 ± 15	13 ± 3
6	**G-II** (10)	72 ± 1	7 ± 0
7	**Aquamet** (5)	66 ± 6	13 ± 1
8	**Aquamet** (10)	66 ± 5	6 ± 1

[a] Substrate concentration: 0.5 mM; [b] results from two independent catalytic runs.

Although the *N*-acetylindole precursor **9a** afforded modest conversion with the biotinylated catalyst **Biot-Ru** (Table 2, entries 1, 2), we screened the RCM activity with artificial metalloenzymes (ArMs) based on the biotin-streptavidin technology. The presence of a biotin anchor on an Hoveyda Grubbs-derived catalyst ensures that, in the presence of equimolar amounts streptavidin (Sav) isoforms, the metathesis catalyst is quantitatively embedded within the Sav host. Site-directed mutagenesis at close-lying residues (e.g. S112 and K121, ref. 13) allows to genetically improve the RCM activity [27-30]. A screening of > 50 Sav mutants at pH 4, 5 and 6 was carried out (see Supporting info, Figure S7-9). Selected results of the RCM activity of substrate **9a** are collected in Table 4. This screening reveals the following trends: i)

The free catalyst **Biot-Ru** performs best at pH = 6 (Table 3, entry 6-8). Up to 47 % yield is achieved with 2.5 mol % **Biot-Ru** (e.g. 19 TON). ii) In contrast, the ArMs perform best at pH 4, affording up to 16 % yield of *N*-acetylindole (Table 3, entry 2-4, 6 TON). iii) Mutations at position K121 have a positive effect on the RCM activity. We hypothesize that removal of the basic lysine residue contributes to lower the local pH around the Ru-center [31]. Accordingly, the ArM **Biot-Ru**·Sav^{K121L} outperforms **Biot-Ru**·Sav^{WT} at pH 6, yielding 18 % (7 TON) of the ring-closed product **7b**.

Table 3. Summary of RCM activity of ArMs **Biot-Ru**·Sav^{K121L} using diolefin **9a**.



9a (2 mM) **7b**

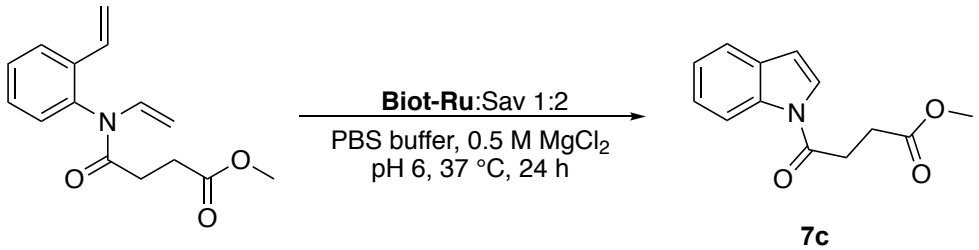
Entry[a]	Sav	pH	Product (μM)[b]	Yield (%)	TON
1	—	4.0	155 ± 7	8 ± 0	3 ± 0
2	WT	4.0	220 ± 2	11 ± 0	4 ± 0
3	K121L	4.0	323 ± 24	16 ± 1	6 ± 1
4	S112N	4.0	282 ± 7	14 ± 0	6 ± 0
5	—	6.0	940 ± 4	47 ± 0	19 ± 0
6	WT	6.0	167 ± 1	8 ± 0	3 ± 0
7	K121L	6.0	359 ± 26	18 ± 3	7 ± 1
8	S112N	6.0	118 ± 10	6 ± 0	2 ± 0

[a] [**9a**] = 2.0 mM in PBS buffer and acetone (5 % V/V); [b] results from two independent catalytic runs.

The catalytic activity of ArMs with the substrate **9b** is collected in Table 4. Again, removal of the lysine in position 121 had a positive effect on RCM, giving a threefold increase in activity: from 7 % yield of indole **7c** with **Biot-Ru**·Sav^{WT} (2 TON) to 18 % with **Biot-Ru**·Sav^{K121A} and 20 % with both **Biot-Ru**·Sav^{K121F} (6 TON) and **Biot-**

Ru·Sav^{K121L} (Table 4, entries 2-5). The free cofactor performed best yielding 26 % of *N*-succinylindole methylester (8 TON, Table 4, entry 1).

Table 4. Summary of RCM activity of ArMs **Biot-Ru**·Sav^{K121L} using diolefin **9b**.



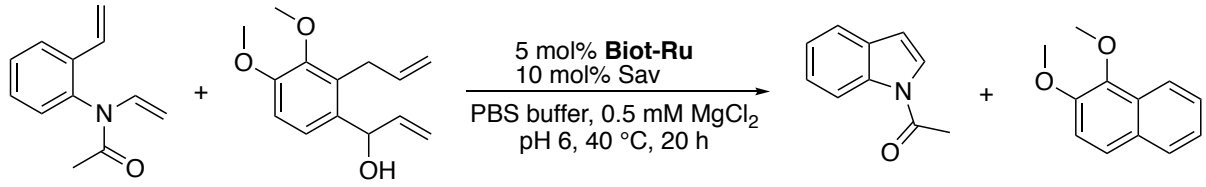
9b (2 mM) **7c**

Entry	Sav	mol % **Biot-Ru**	Yield (%)	TON
1	—	3.33	26.0	8
2	WT	3.33	7.0	2
3	K121A	3.33	18.0	5
4	K121F	3.33	20.0	6
5	K121L	3.33	20.0	6

Next, we tested the influence of the presence of an additional diolefinic substrate **10** on the RCM activity. For this purpose, the RCM of the *N*-acetylindole precursor **9a** was carried out in the presence or absence of diolefin **10**, while keeping the overall catalyst concentration at 5 mol %. Catalysis was performed either with the free catalyst **Biot-Ru** or with the ArMs **Biot-Ru**·Sav^{WT} and **Biot-Ru**·Sav^{K121L}. Unexpectedly, the yield of *N*-acetylindole **7b** increased when the catalysis was performed in the presence of both substrates **9a** and **10**. The free cofactor gave 71 % yield of *N*-acetylindole **7b** compared to 38 % yield when using substrate **9a** alone (Table 5, entry 1-3). This difference in yield was not noticed in previous RCM experiments with substrate **9a** alone, not even when the catalyst concentration was increased from 5 % to 10 %

(Table 2, entry 1-2). We hypothesize that the presence of a second and more reactive substrate delays the catalyst decomposition, probably due to the suppression of the Ru methylidene species which is known to be highly unstable [32-35]. This effect occurs also with the ArMs, producing over sixfold increase in yield with **Biot-Ru**·Sav^{WT} (Table 5, entry 4-6). The ArM **Biot-Ru**·Sav^{K121L} produced an increase in yield from 11 % to 42 % (Table 5, entry 7-9). The influence of the pH on this competition assay was evaluated with selected Sav mutants at pH 4,5 and 6 (see supporting info, Figure S10). We finally evaluated the aqueous RCM of the substrate **10** alone using the same conditions. Strikingly, in the presence of **9a**, the yield of **11** drops from 20 % to 11 % (Table 5, entry 5-6) with **Biot-Ru**·Sav^{WT} and from 65 % to 39 % with **Biot-Ru**·Sav^{K121L} (Table 5, entry 8-9). In contrast, the free catalyst **Biot-Ru** improves the yield of **11** from 35 % to 57 %, suggesting that the ArMs preferentially lead to higher yields of *N*-acetylindole **7b** from an equimolar mixture of substrates **9a** and **10**.

Table 5. Summary of the results of competition RCM of substrates **9a** and **10** in the presence of ArMs.



Entry	Sav	Substrate (mM)	Yield (%) **7b**	Yield (%) **11**	TON (**7b**)	TON (**11**)
1		**9a** (0.4)	38 ± 0	—	7.6 ± 0.0	—
2	—	**10** (0.4)	—	35 ± 0	—	7.0 ± 0.0
3		**9a** (0.2) + **10** (0.2)	71 ± 0	57 ± 0	7.1 ± 0.0	5.7 ± 0.0
4	WT	**9a** (0.4)	2 ± 0	—	< 1	—
5		**10** (0.4)	—	20 ± 0	—	4.0 ± 0.0

6		**9a** (0.2) + **10** (0.2)	13 ± 3	11 ± 3	1.3 ± 0.3	1.1 ± 0.3
7		**9a** (0.4)	10 ± 1	—	2.0 ± 0.2	—
8	K121L	**10** (0.4)	—	65 ± 0	—	13.0 ± 0.0
9		**9a** (0.2) + **10** (0.2)	42 ± 0	39 ± 0	4.2 ± 0.0	4.0 ± 0.0

Conclusion

In conclusion, we report herein the synthesis of *N*-substituted indoles via aqueous RCM of *N*-vinylanilide derivatives. RCM with commercially available catalysts yielded up to 72 % of *N*-acetylindole in PBS buffer at pH = 6 and 2-5 % (V/V) organic cosolvents. In the presence of ArMs based on the biotin-streptavidin technology, up to 42 % of *N*-acetylindole was achieved. Interestingly, the yield of *N*-acetylindole could be improved by addition of a metathesis substrate in the reaction medium, revealing that the addition of a second metathesis substrate has a beneficial effect on the RCM of the indole precursor.

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Conflict of interest The authors declare that they have no conflict of interest.

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