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Biotinylated Metathesis Catalysts: Synthesis and Performance in Ring Closing Metathesis

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Abstract Nine biotinylated Grubbs–Hoveyda and Grubbs-type metathesis catalysts were synthesized and evaluated in ring closing metathesis reactions of *N*-tosyl diallylamine and 5-hydroxy-2-vinylphenyl acrylate. Their catalytic activity in organic- and aqueous solvents was compared with the second generation Grubbs–Hoveyda catalyst. The position of the biotin-moiety on the *N*-heterocyclic carbene was found to critically influence the catalytic activity of the corresponding ruthenium-based catalysts.

Keywords Fluorescence assay · Aqueous catalysis · Ring closing metathesis · Coumarin · Hoveyda–Grubbs

1 Introduction

In the past 40 years, the olefin metathesis reaction has emerged and matured as a very efficient and elegant method for formation of C=C double bonds [1–6]. It is widely used not only in small-scale laboratory research, but also in large-scale industrial production [7, 8]. Among the frontiers in olefin metathesis, one should mention: (i) control of *E*/*Z* selectivity [9–13], (ii) enantioselective ring-closing metathesis (RCM hereafter) [14–18], (iii) the application of metathesis for the conversion of biomass into useful products [19–24], and metathesis as a bioorthogonal ligation tool [25–27]. Despite these great achievements, aqueous-phase metathesis remains a challenge [28–36].

To address this challenge, we and others have relied on the creation of artificial metalloenzymes for olefin metathesis [37–40]. For this purpose, a catalytically competent Hoveyda–Grubbs moiety is anchored within a protein scaffold to afford an artificial metathesase. In this context, we and others have been exploiting the biotin–streptavidin technology to create artificial metalloenzymes for a variety of transformations [41–44], including hydrogenation [45], transfer hydrogenation [46], allylic alkylation [47], benzannulation [48], sulfoxidation [49], dihydroxylation [50] as well as olefin metathesis [37–40]. Traditionally, a chemogenetic optimization scheme is used to improve catalytic performance of artificial metalloenzymes. Genetic diversity is created by introducing point mutations on the streptavidin gene. Chemical diversity is achieved upon varying the position of the biotin anchor and the spacer between the anchor and the Ru-moiety. Previous experience in artificial metalloenzymes clearly demonstrates that catalytic activity is critically dependent on the first coordination sphere around the active metal and thus requires screening a variety of different catalyst precursors. With this goal in mind, we report on the synthesis and evaluation of the catalytic performance of nine biotinylated metathesis catalysts.

2 Results and Discussion

We previously reported on an artificial metathesase relying on combining either avidin or streptavidin with either **Biot-1** or **Biot-*m*-ABA-1** [37]. In this study, we set out to vary the position of the enantiopure biotin-moiety and to

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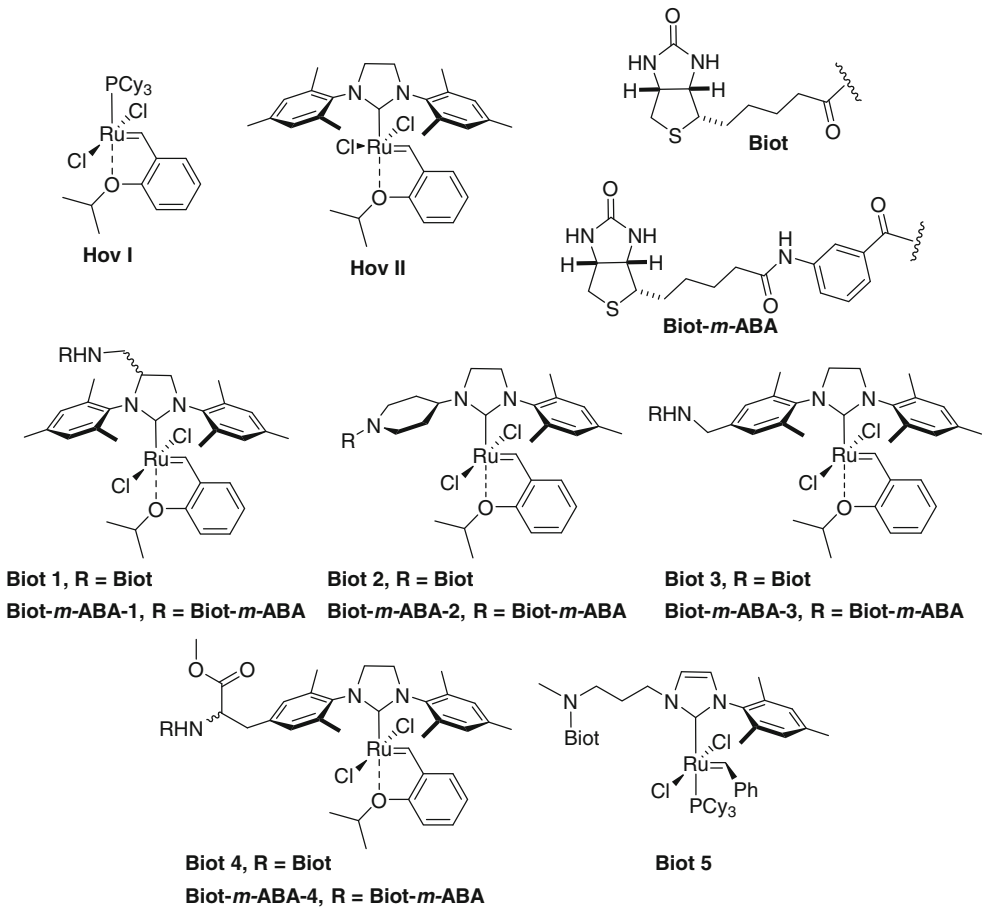


Fig. 1 Biotinylated metathesis catalysts used in this study

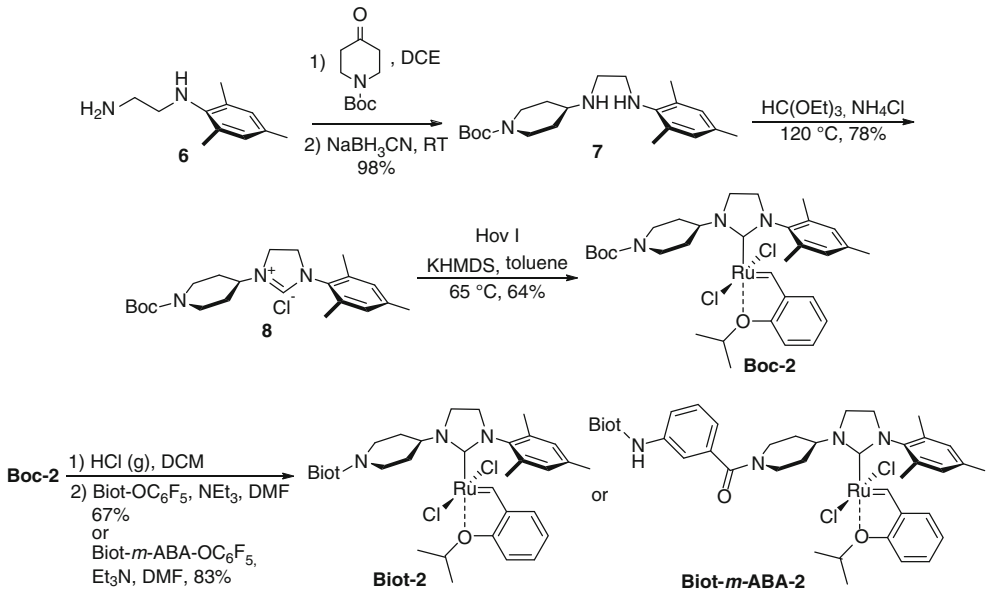
identify the most promising catalyst under various catalytic conditions. All complexes tested in this study are presented in Fig. 1. For complexes **Biot-1** or **Biot-*m*-ABA-1** and **Biot-4** or **Biot-*m*-ABA-4**, the presence of an asymmetric carbon between the enantiopure biotin anchor and the *N*-heterocyclic carbene moiety, leads to diastereomeric mixtures. No effort was made to separate these prior to catalytic evaluation.

While one *N*-mesityl substituent is critical for high catalytic activity [51], we set out to link the biotin anchor on the other *N*-substituent of the *N*-heterocyclic carbene moiety. We evaluated both aromatic (e.g. **Biot-3**, **Biot-*m*-ABA-3**, and **Biot-4**, **Biot-*m*-ABA-4**), as well as aliphatic substituents (e.g. **Biot-2**, **Biot-*m*-ABA-2**, and **Biot-5**) at this position.

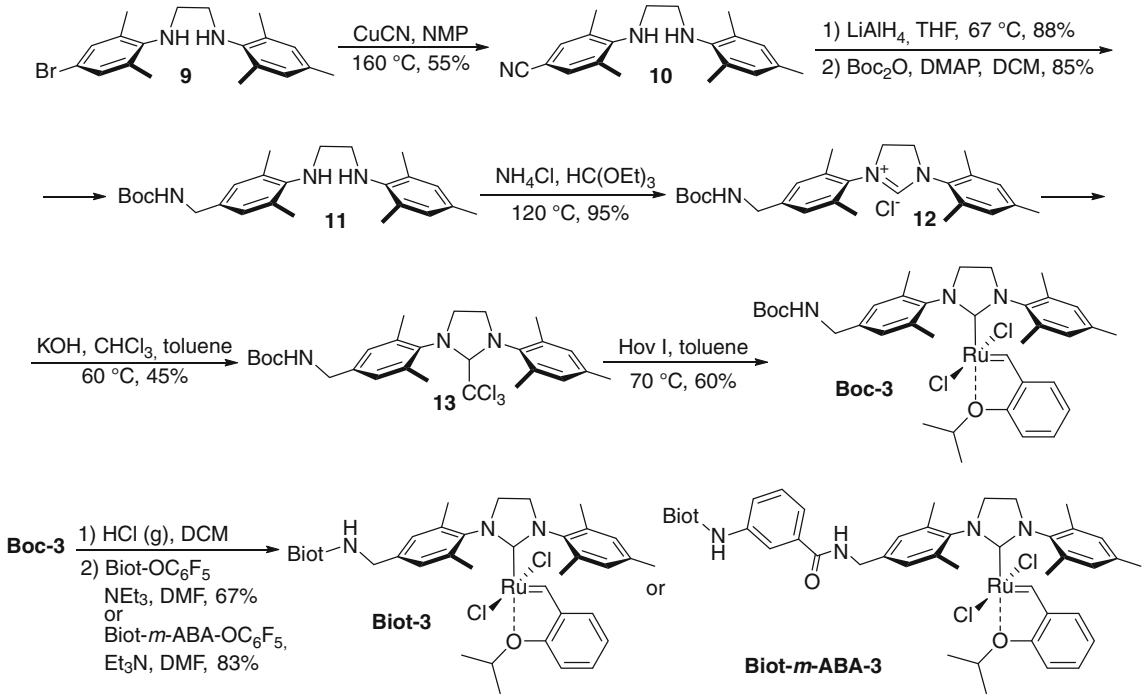
With this aim, we set out to prepare the key Boc-protected imidazolium intermediate **Boc-2**. Reductive amination of the alkylated aniline **6** [52] yielded the 1,2-diamine **7**. Cyclisation with triethyl orthoformate provided the imidazolium salt **8**. Ligand exchange with Hoveyda–Grubbs first generation catalyst **Hov I** afforded the Boc-protected ruthenium NHC complex **Boc-2**. Deprotection with gaseous hydrogen chloride and coupling with either activated biotin or activated biotin *m*-aminobenzoic acid afforded **Biot-2** and **Biot-*m*-ABA-2** respectively (Scheme 1).

The next biotinylated catalysts included two mesityl groups with a biotin linked to one of these. For this purpose, we used the procedure reported by Gilbertson et al. relying on the unsymmetrical diamine **9** [53]. Rosenmund-von Braun cyanation followed by reduction and Boc-protection yielded compound **11**. This latter was reacted with triethylorthoformate in the presence of ammonium chloride to provide the imidazolium salt **12**. Reaction of **12** with chloroform and KOH yielded the chloroform adduct **13** [54]. Ligand exchange with **Hov I** catalyst provided the ruthenium NHC complex **Boc-3** which, after Boc-deprotection, was reacted with activated biotin and biotin *m*-aminobenzoic acid to afford **Biot-3** and **Biot-*m*-ABA-3** respectively (Scheme 2).

To evaluate the influence of the spacer length between the biotin-anchor and the NHC, we synthesized another type of catalysts (**Biot-4** and **Biot-*m*-ABA-4**) which bear an additional carbon when compared to **Biot-3** and **Biot-*m*-**



Scheme 1 Synthesis of the biotinylated complexes **Biot-2** and **Biot-*m*-ABA-2**

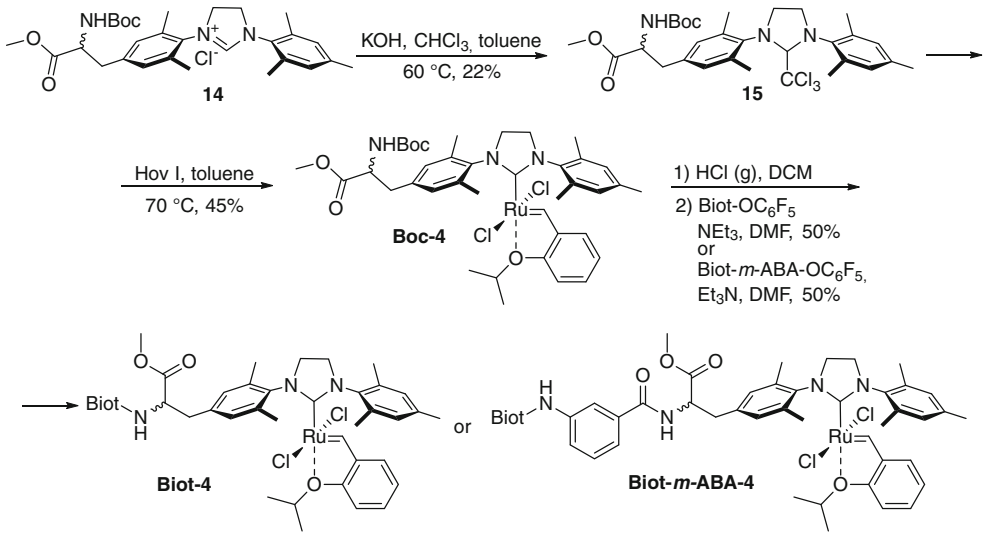


Scheme 2 Synthesis of the biotinylated complexes **Biot-3** and **Biot-*m*-ABA-3**

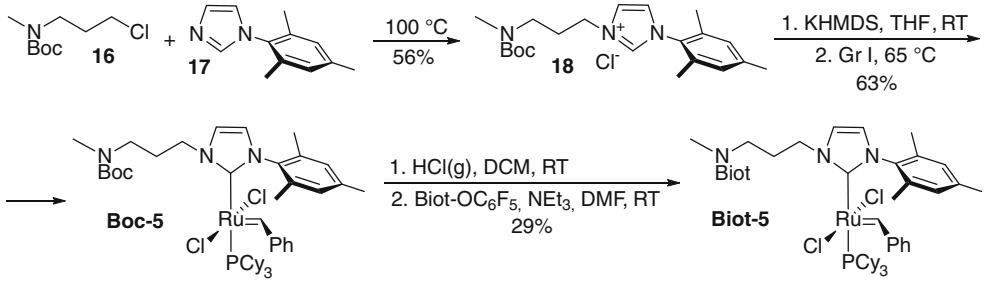
ABA-3. The NHC ligand **14** was synthesized according to a published procedure [53]. Preparation of the chloroform adduct followed by reaction with **Hov I** yielded the ruthenium NHC complex **Boc-4**. After Boc deprotection and reaction with activated biotin derivatives, **Biot-4** and **Biot-m-ABA-4** were obtained (Scheme 3).

Finally, the Grubbs type catalyst **Biot-5** was prepared. The Boc-protected (3-chloropropyl)-*N*-methyl amine **16** was reacted with *N*-mesitylimidazole **17** to produce the imidazolium salt **18**. Reaction of the carbene, generated in situ from **18** in the presence of KHMDS, with the Grubbs first generation catalyst gave complex **Boc-5**. After deprotection of the amino group by HCl and reaction with activated biotin, the desired **Biot-5** was obtained (Scheme 4).

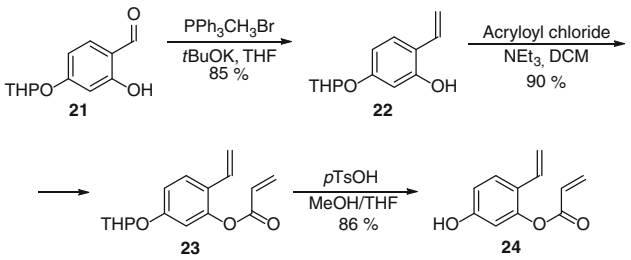
The performance of the biotinylated catalysts was evaluated in the ring closing metathesis of two model



Scheme 3 Synthesis of the biotinylated complexes **Biot-4** and **Biot-*m*-ABA-4**



Scheme 4 Synthesis of the biotinylated complex **Biot-5**



Scheme 5 Synthesis of coumarin precursor **24**, substrate for RCM reaction [56–59]

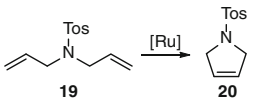
substrates: the diallyl tosylamide **19** and the coumarin precursor **24**. The second substrate was produced from aldehyde **21** [55] via a Wittig reaction followed by esterification with acryloyl chloride and deprotection of the hydroxy group (Scheme 5).

The activity of the new biotinylated precatalysts was compared with that of second-generation Grubbs–Hoveyda complex **Hov II**. The results for RCM reaction of diallyl tosylamide **19** are summarized in Table 1 and Fig. 2 and results for RCM reaction of coumarin precursor **24** are presented in Table 2 and on Fig. 3. All RCM reactions were analysed by reversed phase HPLC and revealed no product isomerization.

As a starting point, we performed the RCM of diallyl tosylamide **19** with 1 mol% biotinylated catalysts and a 0.1 M substrate concentration at 37 °C in dichloromethane. The results reveal that most of biotinylated catalysts with two mesityl moieties bound to imidazoline ring, viz. **Biot-1**, **Biot-*m*-ABA-1** and **Biot-*m*-ABA-4** exhibit the same activity as the parent **Hov II** catalyst and reached almost full conversion. Similar results were also observed for structurally related complexes, namely **Biot-3**, **Biot-*m*-ABA-3** and **Biot-4** with conversion around 70–80 %. These results are consistent with previous observations suggesting that catalysts containing two bulky aromatic substituents in the NHC ligand have the highest activity and stability. Accordingly, **Biot-2** and **Biot-*m*-ABA-2**, are less active. In stark contrast, **Biot-5** is a good RCM catalyst, affording product **20** in 85 % yield.

Upon decreasing the substrate concentration to 0.01 M, similar trends were observed. The presence of a biotin moiety led to marked decrease in conversion, especially

Table 1 Comparison of activity of catalysts in RCM of diallyl tosylamide **19**



Entry	Catalyst[a]	Yield (%)[b]	Yield (%)[c]	Yield (%)[d]
1	**Hov II**	>99	>99	75
2	**Biot-1**	93	83	13
3	**Biot-*m*-ABA-1**	96	76	28
4	**Biot-2**	43	18	1
5	**Biot-*m*-ABA-2**	41	19	1
6	**Biot-3**	79	70	17
7	**Biot-*m*-ABA-3**	76	49	7
8	**Biot-4**	69	35	12
9	**Biot-*m*-ABA-4**	97	84	10
10	**Biot-5**	85	21	2

Experiments were performed in triplicate (yields ±2 %). See SI for experimental details

[a] 1 mol% of [**Ru**], 37 °C, 24 h

[b] CH_2Cl_2, [**19**] = 0.1 M

[c] CH_2Cl_2, [**19**] = 0.01 M

[d] H_2O/DMSO 84:16, [**19**] = 0.1 M

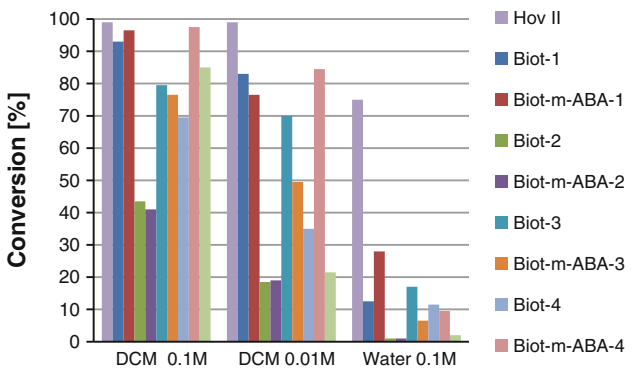
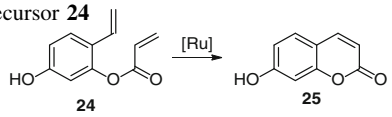


Fig. 2 Comparison of catalysts activity in RCM reaction of diallyl tosylamide

with complexes bearing an alkyl substituent on the NHC (e.g. **Biot-2** and **Biot-*m*-ABA-2** and **Biot-5**).

Next, the reactions were performed in a water:DMSO, 84:16 mixture (DMSO was required to dissolve both substrate and catalyst as both substrates and catalysts exhibit very low solubility in pure water). Except for **Hov II** (75 % yield), the yields were dramatically lower. The best biotinylated catalyst, **Biot-*m*-ABA-1**, reached only ca. 30 % yield. We speculate that the low yields may be due to the limited catalyst's stability in a water. During the reactions, all reaction mixtures changed colour from green (Hoveyda-type catalysts) or purple (Grubbs-type catalyst) to brownish. The biotinylated catalysts also decompose slowly in

Table 2 Comparison of activity of catalysts in RCM reaction of coumarin precursor **24**



Entry	Catalyst[a]	Yield (%)[b]	Yield (%)[c]
1	**Hov II**	>99	4
2	**Biot-1**	97	1
3	**Biot-*m*-ABA-1**	70	3
4	**Biot-2**	99	1
5	**Biot-*m*-ABA-2**	88	1
6	**Biot-3**	50	4
7	**Biot-*m*-ABA-3**	48	1
8	**Biot-4**	52	1
9	**Biot-*m*-ABA-4**	50	1
10	**Biot-5**	22	1

See SI for experimental details

[a] 5 mol% of [**Ru**], 37 °C, 24 h; experiments performed in triplicate

[b] CH_2Cl_2, [**24**] = 0.025 M (yield ± 5 %)

[c] H_2O/DMSO 84:16, [**24**] = 0.025 M

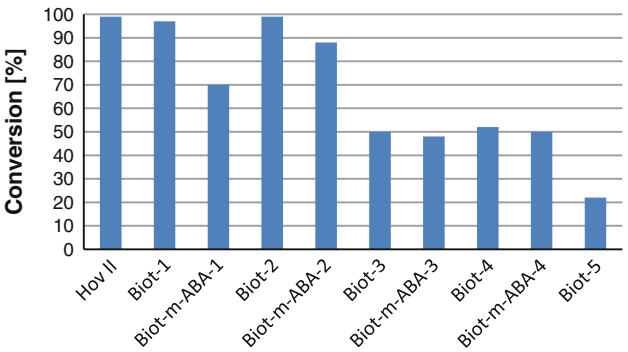


Fig. 3 Comparison of catalysts activity in RCM reaction of 5-hydroxy-2-vinylphenyl acrylate **24** in CH_2Cl_2

DCM. However, as the RCM reaction is significantly faster in DCM, the decomposition does not affect the yields as much.

As the umbelliferone precursor **24** bears an electron withdrawing olefinic moiety which decreases its reactivity, the corresponding RCM reactions were performed with 5 mol% biotinylated catalyst (Table 2) in 0.025 M concentration at 37 °C in dichloromethane. Under these conditions, the two biotinylated catalysts, **Biot-1** and **Biot-2** reached near quantitative conversions. The other biotinylated Hoveyda-type catalysts gave moderate yields approaching 50 %. The Grubbs type Grubbs-type catalyst **Biot-5**, gave only 22 % yield. When reactions were performed in a water:DMSO mixture, <5 % conversion was obtained for all catalysts, including **Hov II**.

In the RCM reaction of diallyl tosylamide **19**, the catalyst **Biot-2** displays only 43 turnovers (TONs, using 1 mol% catalyst loading). For the more challenging substrate **24**, 20 TONs were obtained (using 5 mol% catalyst loading). We speculate that this is the result of a delicate balance between catalyst's activity and its decomposition: the best TON is observed for the challenging substrate **24**, but only a modest TON is obtained for the standard diallyltosylamide substrate **19** before the catalyst becomes inactivated.

N,*N*-diallyl tosylamine **19** has been used as a benchmark for aqueous RCM reactions by several research groups. In most cases, 5 mol% either of classic (e.g. **Gr II**, **Hov II**) or water soluble derivatives were used in water:organic solvent mixtures (i.e. EtOH, DME, MeOH and DMSO). Among the catalysts tested, for a reaction performed in the mixture DME:water (2:1), the **Gr II** gives near quantitative conversions (up to 100 TONs, using 1 mol% catalyst) [31]. Quantitative conversions were also obtained with Raines' [60] or Blechert's [61] catalysts, albeit using 5 mol% catalyst. Reactions in pure water were performed, mainly with 5 mol% catalyst. In these cases however, either classic **Gr I** and **Gr II** were used in the presence of surfactants [62, 65], calix[*n*]arenes [63] or dendrimers [64].

In comparison, the biotinylated catalysts presented herein gave lower or comparable results. It is however difficult to strictly compare these as the reactions are seldom carried under the exact same conditions.

3 Conclusion

In this study, a series of biotinylated ruthenium-based catalysts was synthesized and their activity was evaluated for two model RCM reactions with either diallyl tosylamide **19** or the umbelliferone precursor **24**. While all biotinylated catalysts performed reasonably well in concentrated dichloromethane, their performance decreased significantly in aqueous solution. We speculate that the presence of a thioether moiety on the biotin anchor may interact with the ruthenium [65], thus hampering catalytic turnover. This is particularly pronounced in aqueous solution and may be traced back to the substitution of a chloride by water followed by coordination of the thioether. For the umbelliferone precursor **24**, modest to good conversions were observed in dichloromethane. In stark contrast, RCM in water does not proceed to any significant level. In the context of artificial metalloenzyme design, this substrate, which upon RCM yields the fluorescent umbelliferone **25**, is thus an ideal candidate to evaluate the hydrophobicity of the catalytic site as we anticipate that the second coordination sphere provided by the protein may lead to significant improvement in conversion.

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