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Artificial metalloenzymes for olefin metathesis based on the biotin-(strept)avidin technology†

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Incorporation of a biotinylated Hoveyda-Grubbs catalyst within (strept)avidin affords artificial metalloenzymes for the ring-closing metathesis of *N*-tosyl diallylamine in aqueous solution. Optimization of the performance can be achieved either by chemical or genetic means.

In recent years, artificial metalloenzymes have emerged as alternative tools to complement organometallic-, enzymatic-, heterogeneous- and organo-catalysis. Artificial metalloenzymes result from incorporation of a catalytically competent organometallic moiety within a macromolecule (protein or DNA).[1] Reactions implemented to date include: hydrogenation, transfer-hydrogenation, allylic substitution, Diels–Alder, Friedel–Crafts, Michael-addition, hydroformylation, hydrolysis, fluorination, dihydroxylation, epoxidation, sulfoxidation, *etc*.[2]

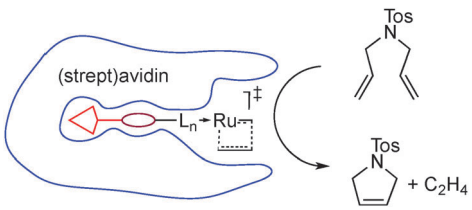
With the advent of chemical biology, transition metal complexes have attracted attention as catalysts for the site-selective, bio-orthogonal modification of proteins.[3] For naturally occurring functionalities (other than cysteines and lysines)[4] the most promising ligation reactions include N-terminal derivatization,[5] tyrosine allylation,[6] tryptophane modification,[7] *etc*.[8] For functionalities absent from proteogenic aminoacid side chains, the Staudinger ligation and the Huisgen [3 + 2] cycloaddition reaction occupy a place of choice as biologically tolerant reactions.[3] More recently, palladium-catalyzed cross-coupling reactions[9] and the olefin metathesis have been scrutinized.[10] Based on our experience in the field of artificial metalloenzymes relying on the biotin avidin technology,[1c] we set out to implement the olefin metathesis in the artificial metalloenzyme repertoire, Scheme 1. In an independent study, Hilvert and coworkers have developed a covalent anchoring strategy to create artificial metalloenzymes for olefin metathesis.[1g]

Thanks to its activity and robustness, we selected the Hoveyda-Grubbs[11] 2nd generation catalyst as a platform for functionalization with a biotin-anchor. For this purpose, complex Boc-**1**,[12]was deprotected *in situ* and allowed to reacted with (+)-biotin **Biot**OC_6F_5 or with ***Biot-m-ABA***OC_6F_5 to afford the corresponding biotinylated metathesis catalysts **Biot-1** and **Biot-*m*-ABA-1**, respectively (Scheme 2 and Supporting Information†).

As a model reaction, we selected the ring-closing metathesis (RCM) of *N*-tosyl diallylamine to afford the corresponding *N*-tosyl-3-pyrrolidine in the presence of 5 mol% ruthenium catalyst. As both catalyst and substrate were insoluble in water, they were dissolved in DMSO, affording a final 5 : 1 water : DMSO ratio in catalysis.

Initial attempts with biotinylated catalyst **Biot-1** and **Biot-*m*-ABA-1** in the presence of streptavidin (Sav hereafter) were disappointing, affording a single turnover at best (Table 1, entries 2, 3). Under otherwise identical conditions but without Sav, **Biot-1** yielded 15 TONs (*i.e*. 74% yield), highlighting the influence of second coordination sphere contacts on catalytic performance (Table 1, entry 1). As suggested by Davis and coworkers, we hypothesized that the sea of functionalities present on the host protein's surface may inhibit the catalyst, thus leading to low turnovers.[16] Addition of $MgCl_2$ (0.5 M) improved the conversion (33% with **Biot-*m*-ABA-1** ⊂ Sav). As an alternative to Lewis acid passivation, catalytic runs were performed at pH 4.0 affording 42% conversion with **Biot-*m*-BA-1** ⊂ Sav. The acetate buffer (0.1 M, pH 4.0) did not inhibit catalysis, suggesting that carboxylates do not interfere with ruthenium. Under all conditions tested, the **Biot-*m*-ABA-1** clearly outperformed **Biot-1** in Sav, which demonstrates that introduction of a spacer between the biotin anchor and the Ru-moiety is an efficient means to optimize chemically the performance of the artificial metalloenzyme.

Genetic optimization was achieved by substitution of streptavidin by avidin (Avi hereafter). Although slightly higher than with Sav as host, the conversion at pH 7.0 remained



Scheme 1 Artificial metalloenzymes for olefin metathesis based on the biotin-avidin technology. Tethering a biotin anchor (red) combined with a spacer (brown oval) on an Hoveyda-Grubbs type catalyst ensures the localization of the metal moiety within either avidin or streptavidin.

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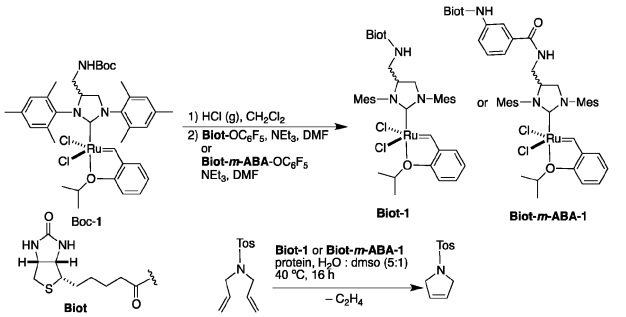

Scheme 2 Synthesis of the biotinylated complexes and operating conditions for ring-closing metathesis.

Table 1 Selected results for the ring-closing metathesis of *N*-tosyl diallylamine[a]

No.	Complex	Protein	pH	$MgCl_2$ [M]	Conv. (%)
1	**Biot-1**	—	7.0	—	74
2	**Biot-1**	Sav	7.0	—	<1
3	**Biot-*m*-ABA-1**	Sav	7.0	—	8
4	**Biot-*m*-ABA-1**	Sav	7.0	0.5	33
5	**Biot-*m*-ABA-1**	Sav	4.0	—	41
6	**Biot-*m*-ABA-1**	Sav	4.0	0.5	71
7	**Biot-1**	Avi	7.0	—	17
8	**Biot-*m*-ABA-1**	Avi	7.0	—	6
9	**Biot-*m*-ABA-1**	Avi	7.0	0.5	54
10	**Biot-1**	Avi	4.0	—	79
11	**Biot-1**	Avi	4.0	0.5	95
12	**Biot-1**	—	4.0	0.5	95

[a] Reaction conditions: $[Sav]_{tetramer}$ 0.25 mM, [catalyst] 0.73 mM, [substrate] 15.21 mM, V_{tot} 120 μL (V_{DMSO} 20 μL), pH 7.0 no buffer; pH 4.0: acetate (0.1 M), 16 h, 40 °C, reactions were carried out in triplicate (See SI for a complete list of all catalytic experiments). Reactions carried out under rigorous exclusion of oxygen gave very similar results.

modest: 17% conversion (*i.e.* > 3 turnovers) with **Biot-1** ⊂ Avi. Upon lowering the pH and/or adding $MgCl_2$, the conversions increased significantly: up to 95% conversion with **Biot-1** ⊂ Avi at pH 4.0 in the presence of $MgCl_2$. The performance of the protein-free catalyst **Biot-1** was also slightly improved by the acidic pH and the presence of $MgCl_2$, affording the RCM product in up to 95% conversion. Overall, **Biot-1** performed better than **Biot-*m*-ABA-1** in the presence of Avi, Table 1 and Fig. 1.

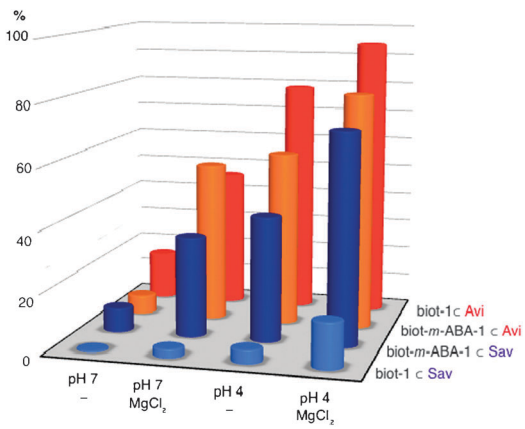

Fig. 1 Graphical summary of the catalytic results obtained for the RCM of *N*-tosyl diallylamine.

In order to exclude that the observed RCM activity is caused by traces of non-(strept)avidin bound catalyst, the affinity of the guest ⊂ host interaction was determined. At pH 7.0, a HABA-substitution titration (HABA: 4′-hydroxyazobenzene-2-carboxylic acid) was carried out:[13] HABA ⊂ Sav and HABA ⊂ Avi display an induced CD signal (λ_{max} 506 nm, log K_a = 3.5 and 5.5, respectively[14]) which, upon addition of **Biot-*m*-ABA-1** decreases up to four equivalents. The resulting CD spectra were analyzed and fitted using specfit (See Fig. S1†).[15] Despite the tetrameric nature of the host protein and the use of a diastereomeric mixture of biotinylated ligands, the data could be fitted with a single binding constant (*i.e.* non-cooperative binding events): log K_a (**Biot-*m*-ABA-1** ⊂ Sav) 8.88 ± 0.6 (pH 7.0, 0.5 M $MgCl_2$) and log K_a (**Biot-1** ⊂ Avi) 8.21 ± 0.053 (pH 7.0). As HABA precipitates at pH 4.0, the **Biot-1** ⊂ Avi affinity was estimated using tryptophane fluorescence quenching[16] (log K_a (**Biot-1** ⊂ Avi) > 9, See Fig. S2†). We conclude that, despite the presence of chaotropic agents and under RCM conditions—using $[host\ protein]_{monomer}$ 1 mM, **Biot-spacer-1** 0.73 mM—the biotinylated metathesis catalyst is quantitatively incorporated (*i.e.* > 99%) either within Sav or Avi, thus confirming that the observed RCM activity is indeed provided by the artificial metalloenzyme.

The results presented herein demonstrate that the biotin-(strept)avidin technology offers a versatile scaffold for the creation of artificial metalloenzymes that display multiple turnovers for ring closing metathesis. Current efforts are directed at harnessing the power of site directed mutagenesis to improve the performance of the hybrid catalyst and to address challenging *enantio*- and diastereoselectivity issues.

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Notes and references

1 (*a*) D. Qi, C.-M. Tann, D. Haring and M. D. Distefano, *Chem. Rev.*, 2001, **101**, 3081–3111; (*b*) Y. Lu, N. Yeung, N. Sieracki and N. M. Marshall, *Nature*, 2009, **460**, 855–862; (*c*) T. R. Ward, *Acc. Chem. Res.*, 2011, **44**, 47–57; (*d*) A. J. Boersma, R. P. Megens, B. L. Feringa and G. Roelfes, *Chem. Soc. Rev.*, 2010, **39**, 2083–2092; (*e*) M. T. Reetz, *Top. Organomet. Chem.*, 2009, **25**, 63–92; (*f*) Q. Jing and R. J. Kazlauskas, *Chem. Cat. Chem.*, 2010, **1**, 953–957.
2 T. Heinisch and T. R. Ward, *Curr. Opin. Chem. Biol.*, 2010, **14**, 184–199.
3 E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974–6998.
4 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, 2nd edn 2008.
5 R. A. Scheck, M. T. Dedeo, A. T. Iavarone and M. B. Francis, *J. Am. Chem. Soc.*, 2008, **130**, 11762–11770.
6 S. Tilley and M. Francis, *J. Am. Chem. Soc.*, 2006, **128**, 1080–1081.
7 (*a*) J. M. Antos, J. M. McFarland, A. T. Iavarone and M. B. Francis, *J. Am. Chem. Soc.*, 2009, **131**, 6301–6308; (*b*) B. V. Popp and Z. T. Ball, *J. Am. Chem. Soc.*, 2010, **132**, 6660–6662.
8 S. D. Tilley, N. S. Joshi and M. B. Francis, *Wiley Encycl. Chem. Biol.*, 2009, **4**, 158–174.
9 J. Chalker, C. Wood and B. Davis, *J. Am. Chem. Soc.*, 2009, **131**, 16346–16347.
10 (*a*) Y. A. Lin, J. M. Chalker, N. Floyd, G. J. L. Bernardes and B. G. Davies, *J. Am. Chem. Soc.*, 2008, **130**, 9642–9643;

(*b*) Y. A. Lin, J. M. Chalker and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 16805–16811; (*c*) Y. A. Lin, J. M. Chalker and B. J. Davis, *Chem. Bio. Chem.*, 2009, **10**, 959–969.

11 (*a*) G. C. Vougioukalakis and R. H. Grubbs, *Chem. Rev.*, 2010, **110**, 1746–1787; (*b*) H. Clavier, K. Grela, A. Kirschning, M. Mauduit and S. Nolan, *Angew. Chem., Int. Ed.*, 2007, **46**, 6786–6801; (*c*) D. Burtscher and K. Grela, *Angew. Chem., Int. Ed.*, 2009, **48**, 442–454; (*d*) A. H. Hoveyda and A. R. Zhugralin, *Nature*, 2007, **450**, 243–251.

12 (*a*) J. P. Jordan and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 2007, **46**, 5152–5155; (*b*) C. Samojłowicz, M. Bieniek and K. Grela, *Chem. Rev.*, 2009, **109**, 3708–3742.

13 M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni and T. R. Ward, *J. Am. Chem. Soc.*, 2004, **126**, 14411–14418.

14 N. M. Green, *Methods Enzymol.*, 1970, **18**, 418–424.

15 (*a*) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 95–101; (*b*) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 257–264; (*c*) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 1133–1139; (*d*) A. Loosli, U. E. Rusbandi, J. Gradinaru, K. Bernauer, C. W. Schläpfer, M. Meyer, S. Mazurek, M. Novic and T. R. Ward, *Inorg. Chem.*, 2006, **45**, 660–668.

16 H. J. Lin and J. F. Kirsch, *Anal. Biochem.*, 1977, **81**, 442–446.