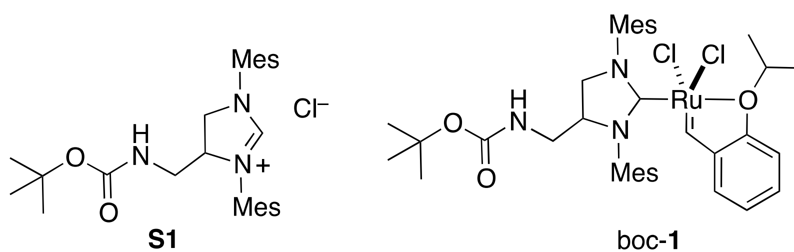
This journal is © The Royal Society of Chemistry 2011

Artificial Metalloenzymes for Olefin Metathesis Based on the Biotin-(Strept)avidin Technology

Cheikh Lo, Mark R. Ringenberg, David Gnandt, Yvonne Wilson and Thomas R. Ward*

Supporting information

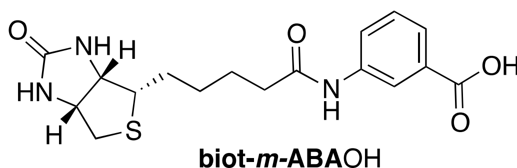
General: ^{1}H, ^{13}C and ^{19}F spectra were obtained on a Bruker 400 MHz and 500 MHz. Chemical shifts are reported in ppm (parts per million). Signals are quoted as s (singlet), d (doublet), t (triplet), and m (multiplet). Electron Spray Ionization Mass Spectras (ESI-MS) were recorded on a Bruker FTMS 4.7T bioAPEX II. Analysis of the catalytic runs was performed on an Agilent 1100 reverse phase HPLC. All solvents were degassed prior to use. Imidazilium salt **S1**, boc-**1**, and *N*-tosyl diallylamine were synthesized as previously described in the literature.[1, 2-4]



Synthesis of biot-1

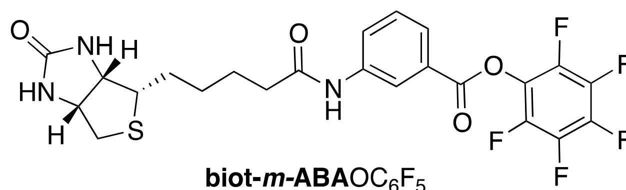
Complex boc-**1** (40 mg, 0.053 mmol) was dissolved in CH_2Cl_2 (2 mL) and HCl gas was bubbled through the solution for 1 h at room temperature. The gaseous HCl was generated by drop-wise addition of concentrated H_2SO_4 to NH_4Cl. The reaction solution was stirred for an additional 2 h at room temperature. The reaction progress was monitored by thin-layer chromatography (TLC) (Hexane/AcOEt 2:1). After the reaction was complete, the solvent was evaporated at reduced pressure and the resulting green solid was dissolved in DMF (2 mL). Biotin pentafluorophenol (22 mg, 0.053 mmol) and Et_3N (0.2 mL, 1.4 mmol) were added to the solution, and the reaction solution was stirred for 16 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified on sephadex LH 20 eluted with CH_2Cl_2 to yield **biot-1** as a green solid (27 mg, 58%). All attempts to separate the diastereomers of **biot-1** were unsuccessful.

^{1}H-NMR (500 MHz, CD_2Cl_2) δ: 16.36 (s, 1H), 7.49 (m, 1H), 7.03 – 6.97 (m, 4H), 6.90 – 6.77 (m, 3H), 4.85 (m, 1H), 4.52 (m, 1H), 4.43 (m, 1H), 4.36 (m, 1H), 4.18 (m, 1H), 3.82 (m, 1H), 3.57 (m, 1H), 3.33 (m, 1H), 3.06 (m, 1H), 2.89 (m, 1H), 2.59 (m, 1H), 2.42 – 1.97 (m, 20H), 1.68 – 1.54 (m, 4H), 1.42 – 1.32 (m, 2H), 1.21 (m, 6H). ^{13}C-NMR (125 MHz, CD_2Cl_2) δ: 174.7, 152.2, 145.0, 143.1 138.7, 136.7, 129.9, 129.8, 122.3, 112.9, 75.2, 63.5, 61.6, 59.9, 55.3, 45.7, 40.5, 35.5, 29.6, 26.6, 25.2, 21.2, 20.5, 17.95. ESI-MS for $C_{42}H_{55}Cl_2N_5O_3RuS$: 846.28 $[M-Cl]^+$.



biot-*m*-ABAOH

The synthesis of **biot-*m*-ABA**OH was carried out as described in the literature.[5]



biot-*m*-ABAOC_6F_5

Synthesis of biot-*m*-ABAOC_6F_5

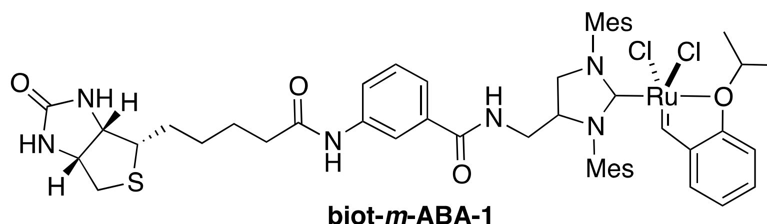
The acid **biot-*m*-ABA**OH (200mg, 1.37 mmol) and *N,N'*-dicyclohexylcarbodiimide (283 mg, 1.37 mmol) were dissolved in DMF (35 mL). A solution of pentafluorophenol (506 mg, 2.75 mmol) in DMF (10 mL) was added and the reaction solution was stirred for 16 h at room temperature.

The reaction mixture was filtered and the solvent evaporated. The resulting solid was washed with hexane (50 mL x 3) to yield **biot-*m*-ABA**OC_6F_5 as a white solid (257 mg, 88%) which was used without further purification

^{1}H NMR (400 MHz, DMSO) δ 10.25 (s, 1H), 8.52 (s, 1H), 7.93 (dt, *J* = 18.0, 9.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.57 (t, *J* = 8.0 Hz, 1H), 6.44 (s, 1H), 6.35 (s, 1H), 4.38 – 4.23 (m, 1H), 4.12 (dd, *J* = 8.9, 3.0 Hz, 1H), 3.18 – 3.04 (m, 1H), 2.90 – 2.68 (m, 1H), 2.56 (d,

Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2011

J = 12.4 Hz, 1H), 2.40 – 2.23 (m, 2H), 1.83 – 1.51 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 172.63, 164.84, 163.58, 142.05, 141.07, 134.54, 130.85, 128.46, 127.14, 126.30, 122.96, 121.08, 61.90, 60.06, 56.25, 40.72, 37.09, 34.21, 30.25, 28.96, 25.82. ^{19}F NMR (376 MHz, DMSO) -153.23 (d, J = 19.6 Hz), -157.28 (t, J = 23.3 Hz), -162.01 (dd, J = 23.2, 19.7 Hz). ESI-MS for $C_{23}H_{20}F_5N_3NaO_4S$: 552.1.



biot-*m*-ABA-1

Synthesis of biot-*m*-ABA-1

Complex **biot-*m*-ABA-1** was synthesized following the same procedure used for the synthesis of **biot-1**, using **biot-*m*-ABA**OC_6F_5 (35 mg, 0.066 mmol) and boc-**1** (50 mg, 0.066 mmol). The crude product was purified on sephadex LH 20 using CH_2Cl_2 as eluent to yield **biot-*m*-ABA-1** as a green solid. (23 mg, 35%). All attempts to separate the diastereomers of **biot-*m*-ABA-1** were unsucessful.

^{1}H-NMR (500 MHz, CD_2Cl_2) δ: 16.38 (s, 1H), δ 7.89 (s, 1H), 7.71 (s, 1H), 7.49 (m, 1H), 7.38 (m, 1H), 7.25 (m, 1H) 7.06 – 6.94 (m, 4H), 6.90 - 6.77 (m, 3H), 4.84 (m, 1H), 4.73 (m, 1H) 4.60 (m, 1H), 4.34 (m, 1H), 4.19 (m, 1H), 3.93 (m, 1H), 3.75 (m, 1H), 3.57 (m, 1H), 3.04 (m, 1H), 2.80 (m, 1H), 2.55 (m, 1H), 2.38 - 2.01 (m, 20H), 1.70 – 1,52 (m, 4H), 1.41 – 1.33 (m, 2H), 1.19 (m, 6H). ^{13}C-NMR (125 MHz, CD_2Cl_2) δ: 152.3, 151.8, 145.1, 142.8, 138.5, 134.4, 131.1, 130.3, 129.9, 129.5, 122.8, 122.3, 118.2, 113.0, 75.2, 63.6, 61.5, 60.6, 55.6, 42.1, 40.5, 36.5, 29.6, 27.9, 27.6, 25.1, 21.0, 20.7, 17.8. ESI-MS for $C_{49}H_{60}Cl_2N_6O_4RuS$: 965.33 [M-Cl]$^+$.

General procedure for ring closing methathesis of *N*-tosyl diallylamine.

This journal is © The Royal Society of Chemistry 2011

A 9 mM stock solution of the complex (either **biot-1** or **biot-*m*-ABAB-1**) was prepared by adding DMSO (50 μL) to an aliquot of the complex (0.36   mol). A 183 mM stock solution of *N*-tosyl diallylamine (27 μL, 91.6   mol in 500 μL DMSO) was prepared in a separate vial. In a pyrex tube was added a 0.25 mM stock solution of protein in buffer or water (100 μL), followed by the addition of the catalyst's stock solution (10 μL) and the mixture was vortexed for one minute. The *N*-tosyl diallylamine (10 μL of the stock solution) was added and the reaction flask was placed in an orbital shaker for 16 h at 40°C. Experiments under rigorous exclusion of oxygen were carried in a glove box and gave identical results.

Upon completion of the reaction, a 15 mM solution of phenylethanol (120 μL, used an internal standard) was added to the reaction vial, followed by MeOH (800 μL). The solution was transferred to an eppendorf tube and centrifuged at 14'000 rpm for 2 minutes. The supernatant (800 μL) was transferred in an HPLC vial and water (800 μL) was added. The sample was subjected to RP-HPLC to determine the conversion.

Experiments in which the concentration of catalyst, $MgCl_2$, and pH were varied were preformed in the same manner, taking care to maintain the same DMSO : water ratio.

HPLC analysis. Column: XDB-C18, Eclipse by Agilent: 150 x 4.6 mm; 5 μm with guard column. Method: $V_{injected}$: 6 μl. Eluent (Solvent A H_2O) , (Solvent B: CH_3CN) 10% B at 0 min, 10% B at 5 min; 90% B at 15 min; 90 % B at 20 min. Detection at 210 nm.

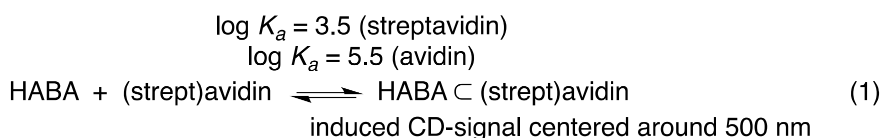
Retention times: Phenylethanol (internal standard): 10.7 min; *N*-tosyl-3-pyrroline: 13.5 min; *N*-tosyl diallylamine: 15.3 min.

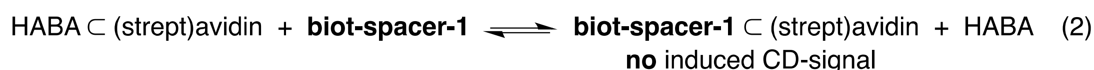
Binding constant determination by CD spectroscopy

In order to determine the affinity of **biot-spacer-1** for (strept)avidin, a substitution titration was carried out using CD-spectroscopy, by following the disappearance of the induced CD-signal of HABA ⊂ (strept)avidin (eq. 1-2). Upon addition of aliquots of the biotinylated complex (either **biot-1** or **biot-*m*-ABA-1**) to HABA ⊂ (strept)avidin, the induced CD-signal

This journal is © The Royal Society of Chemistry 2011

caused by the supramolecular inclusion complex HABA ⊂ (strept)avidin decreases, thus allowing to follow the exchange of HABA for either **biot-1** or **biot-*m*-ABA-1**. The resulting CD data were analyzed and fitted using Specfit as described elswhere.[6] This substitution titration offer the advantage that the curve used for fitting corresponds to the K_{exch} (eq. 2). Thanks to this procedure, significantly higher overall binding affinities $K_{tot} = K_a \cdot K_{exch}$ can be fitted reliably.





(Strept)avidin (8 M initial concentration in Tris-HCl buffer pH = 7, 2.4mL, 19.2 nmol) were charged with a large excess of HABA (9.6 mM stock solution in Tris-HCl buffer pH = 7, 50- and 150 equivalents relative to Avi and Sav tetramer respectively). Aliquots of solutions of **Biot-1** or **Biot-*m*-ABA-1** (0.96 mM in MeOH) were added to HABA ⊂ avidin in 0.25 equiv. steps (5 μL) up to 7 equivalents relative to the monomer. The CD spectra (450 – 600 nm, band width 1 nm, 30 nm/min, 3 accumulations) were recorded after stirring (5 min at room temperature).

This journal is © The Royal Society of Chemistry 2011

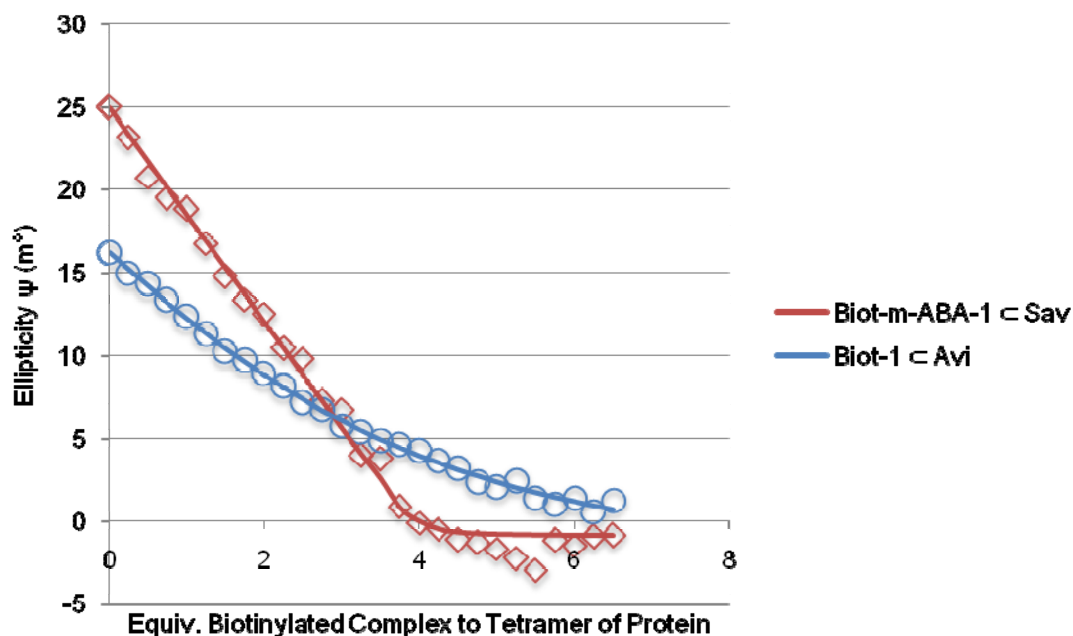


Figure S1. Measured (open symbols) and fitted (solid lines) ellipticity profiles at 530 nm for the HABA ⊂ (strept)avidin substitution titration by **Biot–*m*-ABA–1** in Sav (pH 7.0, 0.5 M $MgCl_2$, red symbols) and **Biot–1** in the presence of Avi (pH 7.0, blue symbols).

Fluorescence Titration of Avidin with Biot-1

A standard solution of Avidin (0.150 μM initial concentration in acetate buffer pH = 4) was treated with **Biot-1** (33 mM, in acetate buffer pH = 4) in ~0.5 equiv. (2 μL) steps to tetrameric Avidin. The concentration of free Avidin was determined by fluorescence quenching, in which the excitation monochromator was set at 290 nm using a bandwidth of 4 nm, and the fluorescence was monitored after each addition at 350 nm using a bandwidth of 12 nm.[7]

This journal is © The Royal Society of Chemistry 2011

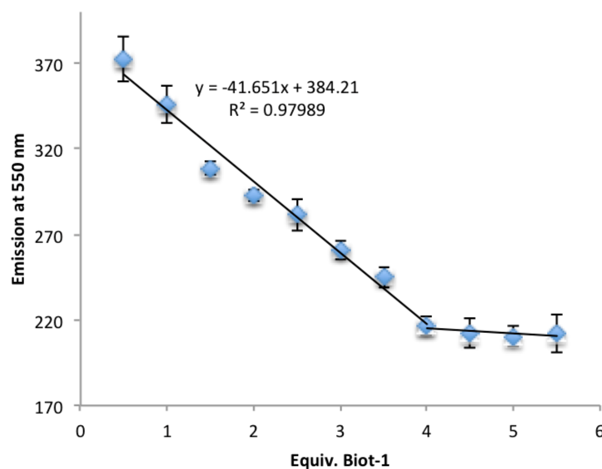


Figure S2. Titration of Avidin with **Biot-1** excitation at 290 nm using a bandwidth of 4 nm, emission monitored at 350 nm using a bandwidth of 12 nm.

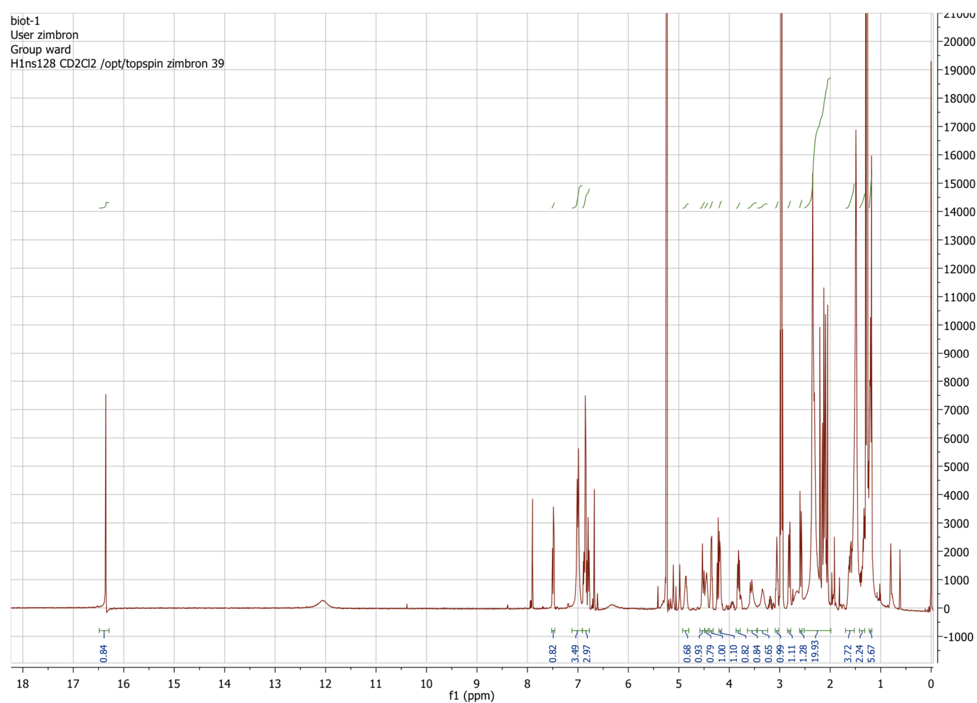


Figure S3. ^{1}H-NMR of **biot-1** in CD_2Cl_2

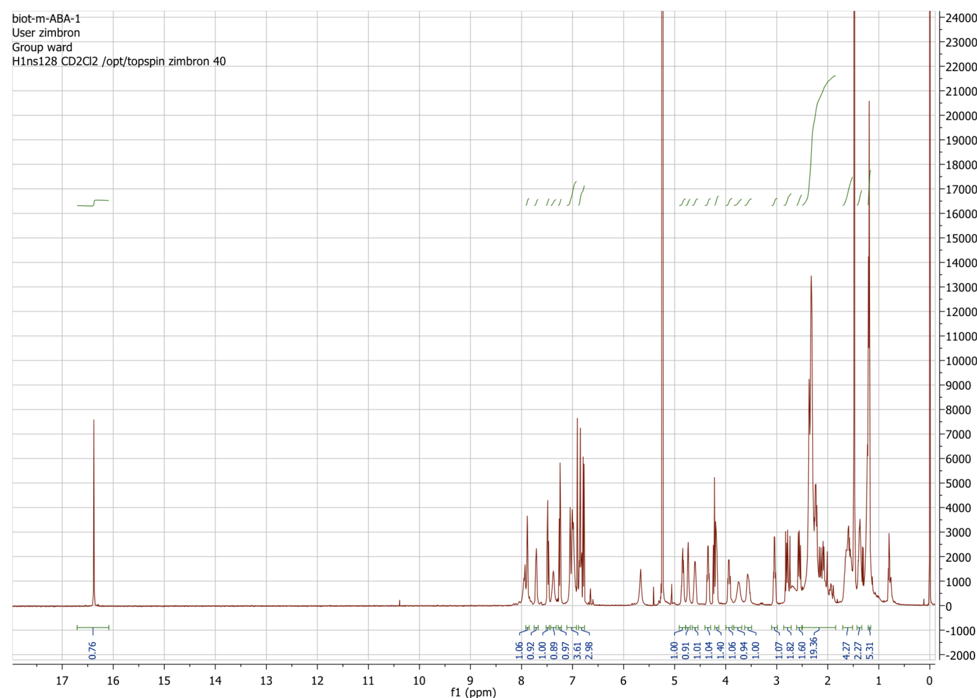


Figure S4. ^{1}H-NMR of **biot-*m*-ABA-1** in CD_2Cl_2

Literature cited

(1) Jordan, J. P.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **2007**, *46*, 5152.

(2) Bieniek, M.; Michrowska, A.; Gulajski, L.; Grela, K. *Organometallics* **2007**, *26*, 1096.

(3) Gessler, S.; Randl, S.; Blechert, S. *Tetrahedron Let.* **2000**, *41*, 9973.

(4) Miyaki, Y.; Onishi, T.; Ogoshi, S.; Kurosawa, H. *J. Organomet. Chem.* **2000**, *616*, 135.

(5) Skander, M.; Humbert, N.; Collot, J.; Gradinaru, J.; Klein, G.; Loosli, A.; Sauser, J.; Zocchi, A.; Gilardoni, F.; Ward, T. R. *J. Am. Chem. Soc.* **2004**, *126*, 14411.

(6) Loosli, A.; Rusbandi, U. E.; Gradinaru, J.; Bernauer, K.; Schlaepfer, C. W.; Meyer, M.; Mazurek, S.; Novic, M.; Ward, T. R. *Inorg. Chem.* **2006**, *45*, 660.

(7) Lin, H.J.; Kirsch, J.F. *Anal. Biochem.* **1977**, *81*, 442