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

Cheikh Lo, Mark R. Ringenberg, David Gnanndt, 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 Spectra (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 $\text{S}_1$, boc-$\text{I}$, and $\text{N}$-tosyl diallylamine were synthesized as previously described in the literature.$^1, 2, 4$

Synthesis of biot-$\text{I}$

Complex boc-$\text{I}$ (40 mg, 0.053 mmol) was dissolved in CH$_2$Cl$_2$ (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$_2$SO$_4$ to NH$_4$Cl. 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$_3$N (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$_2$Cl$_2$ to yield biot-$\text{I}$ as a green solid (27 mg, 58%). All attempts to separate the diastereomers of biot-$\text{I}$ were unsuccessful.
\[ ^1H\text{-NMR (500 MHz, CD}_2\text{Cl}_2) \delta: 16.36 (s, 1H), 7.49 (m, 1H), 7.03 - 6.97 (m, 4H), 6.90 - 6.77 (m, 3H), 4.85 (m, 1H), 4.52 (m, 1H), 4.43 (m, 1H), 4.36 (m, 1H), 4.18 (m, 1H), 3.82 (m, 1H), 3.57 (m, 1H), 3.33 (m, 1H), 3.06 (m, 1H), 2.89 (m, 1H), 2.59 (m, 1H), 2.42 - 1.97 (m, 20H), 1.68 - 1.54 (m, 4H), 1.42 - 1.32 (m, 2H), 1.21 (m, 6H). \]

\[ ^13C\text{-NMR (125 MHz, CD}_2\text{Cl}_2) \delta: 174.7, 152.2, 145.0, 143.1, 138.7, 136.7, 129.9, 129.8, 122.3, 112.9, 63.5, 61.6, 59.9, 55.3, 45.7, 40.5, 35.5, 29.6, 26.6, 25.2, 21.2, 20.5, 17.95. \]

ESI-MS for C\text{42H55Cl2N5O3RuS}: 846.28 [M-Cl]+.

The synthesis of \textit{biot-m-ABA}OH was carried out as described in the literature.\textsuperscript{5}

\[ \text{Synthesis of biot-m-ABA}OC_6F_5 \]

The acid \textit{biot-m-ABA}OH (200mg, 1.37 mmol) and \textit{N,N'}-dicyclohexylcarbodiimide (283mg, 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 \textit{biot-m-ABA}OC\text{6F}_5 as a white solid (257 mg, 88%) which was used without further purification.

\[ ^1H\text{ NMR (400 MHz, DMSO)} \delta 10.25 (s, 1H), 8.52 (s, 1H), 7.93 (dt, J = 18.0, 9.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.57 (t, J = 8.0 Hz, 1H), 6.44 (s, 1H), 6.35 (s, 1H), 4.38 - 4.23 (m, 1H), 4.12 (dd, J = 8.9, 3.0 Hz, 1H), 3.13 - 3.04 (m, 1H), 2.90 - 2.68 (m, 1H), 2.56 (d, 1H). \]
J = 12.4 Hz, 1H), 2.40 – 2.23 (m, 2H), 1.83 – 1.51 (m, 6H). \(^{13}\)C NMR (101 MHz, DMSO) \(\delta\) 172.63, 164.84, 163.58, 142.05, 141.07, 134.54, 130.85, 129.46, 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\(_{5}\)N\(_{3}\)NaO\(_{4}\)S: 552.1.

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-\(m\)-ABAOC\(_{6}\)F\(_{5}\) (35 mg, 0.066 mmol) and boc-1 (50 mg, 0.066 mmol). The crude product was purified on sephadex LH 20 using CH\(_{2}\)Cl\(_{2}\) 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 unsuccessful.

\(^{1}\)H-NMR (500 MHz, CD\(_{2}\)Cl\(_{2}\)) \(\delta\): 16.38 (s, 1H), \(\delta\) 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\(_{2}\)Cl\(_{2}\)) \(\delta\): 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\(_{2}\)N\(_{6}\)O\(_{4}\)RuS: 965.33 [M-Cl]+.

General procedure for ring closing methathesis of N-tosyl diallylamine.
A 9 mM stock solution of the complex (either biot-1 or biot-\textit{m-ABAB}-1) was prepared by adding DMSO (50 \( \mu \)L) to an aliquot of the complex (0.36 mol). A 183 mM stock solution of \( N\)-tosyl diallylamine (27 \( \mu \)L, 91.6 mol in 500 \( \mu \)L 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 \( \mu \)L), followed by the addition of the catalyst’s stock solution (10 \( \mu \)L) and the mixture was vortexed for one minute. The \( N\)-tosyl diallylamine (10 \( \mu \)L 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 \( \mu \)L, used an internal standard) was added to the reaction vial, followed by MeOH (800 \( \mu \)L). The solution was transferred to an eppendorf tube and centrifuged at 14'000 rpm for 2 minutes. The supernatant (800 \( \mu \)L) was transferred to an HPLC vial and water (800 \( \mu \)L) 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 \( \mu \)m with guard column. Method: \( V_{\text{injected}}\): 6 \( \mu \)L. Eluent (Solvent A H\(_2\)O), (Solvent B: CH\(_3\)CN) 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 \( \subset\) (strept)avidin (eq. 1-2). Upon addition of aliquots of the biotinylated complex (either biot-1 or biot-\textit{m-ABA}-1) to HABA \( \subset\) (strept)avidin, the induced CD-signal
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 elsewhere. This substitution titration offer the advantage that the curve used for fitting corresponds to the $K_{\text{exch}}$ (eq. 2).

Thanks to this procedure, significantly higher overall binding affinities $K_{\text{tot}} = K_a \cdot K_{\text{exch}}$ can be fitted reliably.

\[
\begin{align*}
\log K_a &= 3.5 \text{ (streptavidin)} \\
\log K_a &= 5.5 \text{ (avidin)} \\
\text{HABA + (strept)avidin} &\rightleftharpoons \text{HABA ⊂ (strept)avidin} \quad (1) \\
\text{induced CD-signal centered around 500 nm}
\end{align*}
\]

\[
\begin{align*}
\text{HABA ⊂ (strept)avidin + biot-spacer-1} &\rightleftharpoons \text{biot-spacer-1 ⊂ (strept)avidin + HABA} \quad (2) \\
\text{no induced CD-signal}
\end{align*}
\]

(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).
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-I in Sav (pH 7.0, 0.5 M MgCl₂, red symbols) and Biot-I 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-I (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.
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$_2$Cl$_2$. 
**Figure S4.** $^1$H-NMR of biot-$m$-ABA-1 in CD$_2$Cl$_2$

**Literature cited**


