Supplementary Material for Chemical Communications

Chemical Optimization of Artificial Metalloenzymes Based on the Biotin-Avidin Technology: (*S*)-Selective and Solvent-Tolerant Hydrogenation Catalysts via the Introduction of Chiral Aminoacid Spacers

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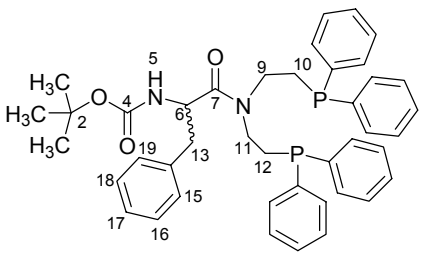
Supplementary Data

Experimental

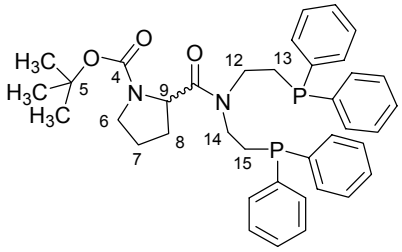
NMR spectra were recorded on Bruker 400 and 500 MHz spectrometers. The ^{1}H, ^{13}C assignements are based on COSY, DEPT and HETCOR experiments. Chemical shifts are reported in ppm (parts per million), relative to external standards TMS (^{1}H, ^{13}C) and H_3PO_4 ^{31}P). The samples were measured in deuterated chloroform (99.8% D) and deuterated dimethylsulfoxide (99.5% D) from Cambridge Isotope Laboratories. Signals were quoted as s (singulet), d (doublet), t (triplet), br (broad), and m (multiplet). Electro-Spray Ionization Mass Spectra (ESI-MS) were recorded on a Finnigan spectrometer using an ion trap. The relative intensities are given in parenthesis (%). The presence of oxidized forms of diphosphine ligands in mass spectra is due to their oxidation during the measurement.

General Procedure for the Synthesis of *tert*-butyl 1-(bis(2-(diphenylphosphino)ethyl)amino)-1-oxo-3-phenylpropan-2-ylcarbamate : Boc-(*R*)- or (*S*)-Phe-1



The diphosphino-amine **H–1**.HCl (Wilson, M. E.; Nuzzo, R. G.; Whitesides, G. M. *J. Am. Chem. Soc.* 1978, **100**, 2269-2270.) (0.10 g, 0.21 mmol), 2-(*tert*-butoxycarbonyl)-3-phenylpropanoic acid (0.05 g, 0.21 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (0.04 g, 0.23 mmol) were mixed in degassed acetonitrile (10 ml) and *N*-methylmorpholine (0.06 ml, 0.52 mmol) was added slowly. The slurry was stirred overnight at room temperature. The solvent was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using hexane / ethyl acetate (9/1) as eluent, allowing isolation of pure product **Boc-Phe-1** (0.09 g, 61%) as white foam. δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.4 (9 H, s, C(*CH*$_3$)), 1.8 (1 H, m, 10-H), 2.0 (1 H, m, 12-H), 2.2 (2 H, m, 10-H, 12-H), 2.8 (2 H, m, 13-H), 2.9 (2 H, m, 9-H), 3.0 (1 H, m, 11-H), 3.5 (1 H, m, 11-H), 4.6 (1 H, dd, *J* 15.6 and 8, 6-H), 5.3 (1 H, d, *J* 8.5, 5-H), 7.0 (2 H, m, 15-H, 19-H), 7.1 (3 H, m, 16-H, 17-H, 18-H) and 7.3-7.4 (20 H, m, Ph); δ_C(126 MHz; $CDCl_3$; Me_4Si) 26.0 (10-C), 27.4 (12-C), 28.3 (1-C), 40.3 (13-C), 44.2 (9-C), 44.8 (11-C), 51.4 (6-C), 79.5 (2-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 154.8 (4-C) and 171.3 (7-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -19.8 and -20.7; m/z (ESI) 689.2 (M^+ + H, 55%), 705.2 (32), 721.1 (100).

General Procedure for the Synthesis of *tert*-butyl 2-((bis(2-(diphenylphosphino)ethyl))carbamoyl)pyrrolidine-1-carboxylate : Boc-(*R*)- or (*S*)-Pro-1



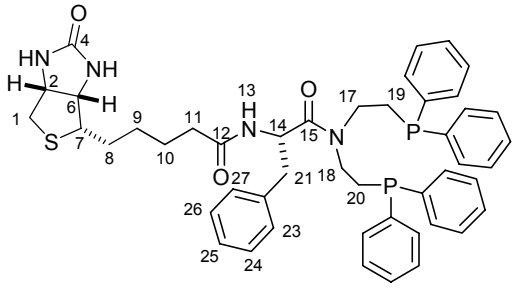
The diphosphino-amine **H–1.**HCl (0.10 g, 0.21 mmol), 1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (0.05 g, 0.21 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (0.04 g, 0.23 mmol) were mixed in degassed acetonitrile (10 ml) and *N*-methylmorpholine (0.06 ml, 0.52 mmol) was added slowly. The slurry was stirred overnight at room temperature. The solvent

was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using hexane / ethyl acetate (9/1) as eluent, allowing isolation of pure product **Boc-Pro-1** (0.10 g, 75%) as white foam. δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.4 (5 H, s, C(*CH₃*)), 1.7 (4 H, s, C(*CH₃*)), 1.6-1.9 (4 H, m, 7-H, 8-H), 2.2-2.5 (4 H, m, 13-H, 15-H), 3.2 (6 H, m, 6-H, 12-H, 14-H), 3.9-4.1 (1 H, m, 9-H), 7.3-7.4 (20 H, m, Ph); δ_C(126 MHz; $CDCl_3$; Me_4Si) 23.3 (7-C), 26.4 (13-C), 26.8 (15-C), 28.5 (1-C), 29.9 (8-C), 44.3 (12-C), 44.9 (14-C), 56.5 (9-C), 79.2 (2-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 153.7 (4-C) and 172.0 (10-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -19.4, -20.0, -20.4 and -20.9; m/z (ESI) 639.2 (M^+ + H, 43%), 655.2 (25), 671.1 (100).

Deprotection of Boc-Amino acid Diphosphine; general procedure:

The Boc protecting group was removed from Boc-AA-1 (AA= Phe or Pro) with 20 equivalents trifluoroacetic acid in dichloromethane (TFA / DCM : 1/2) in the presence of 2 equivalents of anisole. The mixture of TFA/DCM/anisole was degassed for 10 min. before adding the Boc-amino acid diphosphine precursors. The reaction was completed after 3 h at room temperature. The solvent was removed *in vacuo* and the crude precipitate was used for the synthesis of the biotinylated ligands.

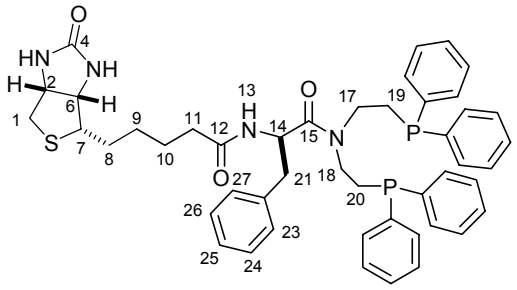
Procedure for the Synthesis of *N*-((*S*)-1-(bis(2-(diphenylphosphino)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)-5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide : Biot-(*S*)-Phe-1



N-*N*-Diisopropylethylamine (0.11 ml, 0.68 mmol) was added to a solution containing biotin pentafluorophenyl ester (0.07 g, 0.17 mmol) and the deprotected amino acid diphosphine H-(*S*)-Phe-1 (0.17 mmol) in dimethylformamide (10 ml). The mixture was stirred 48 h at room

temperature. The solvent was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using chloroform / methanol (25/1) as eluent, allowing isolation of pure product **Biot-(*S*)-Phe-1** (0.05 g, 34%) as a white foam. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.3 (2 H, m, 9-H, 8-H), 1.6 (4 H, m, 8-H, 9-H, 10-H), 1.8 (1 H, m, 19-H), 2.0 (1 H, m, 20-H), 2.2 (3 H, m, 11-H, 20-H), 2.4 (1 H, m, 19-H), 2.7 (1 H, d, *J* 12.8, 1-H), 2.8 (3 H, m, 1-H, 21-H), 3.0 (1 H, m, 18-H), 3.1 (3 H, m, 17-H, 7-H), 3.5 (1 H, m, 18-H), 4.3 (1 H, dd, *J* 8.0 and 4.8, 6-H), 4.5 (1 H, m, 2-H), 4.8 (1 H, dd, *J* 8.0 and 7.6, 14-H), 6.9 (2 H, m, 23-H, 27-H), 7.1 (3 H, m, 24-H, 25-H, 26-H), 7.3-7.4 (20 H, m, Ph) and 7.7 (1 H, br, 13-H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 26.0 (10-C), 26.6 (20-C), 27.5 (19-C), 28.2 (8-C), 28.4 (9-C), 35.9 (11-C), 39.4 (21-C), 40.7 (1-C), 44.6 (18-C), 45.7 (17-C), 50.7 (14-C), 55.8 (7-C), 60.8 (2-C), 62.5 (6-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 165.0 (4-C), 172.5 (15-C) and 173.4 (12-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -20.4; m/z (ESI) 837.4 (M^+ + Na, 100%), 869.5 (22), 853.3 (30).

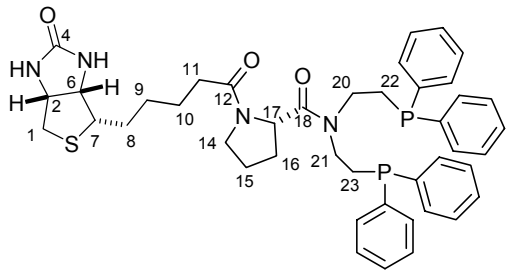
Procedure for the Synthesis of *N*-((*R*)-1-(bis(2-(diphenylphosphino)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)-5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide : Biot-(*R*)-Phe-1



N-*N*-Diisopropylethylamine (0.35 ml, 2.10 mmol) was added to a solution containing biotin pentafluorophenyl ester (0.22 g, 0.53 mmol) and the deprotected amino acid diphosphine H-(*R*)-Phe-1 (0.35 mmol) in dimethylformamide (10 ml). The mixture was stirred 48 h at room temperature. The solvent was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using chloroform / Methanol (25/1) as eluent, allowing isolation of pure product **Biot-(*R*)-Phe-1** (0.17 g, 40%) as a white foam. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.2-1.3 (2 H, m, 9-H, 8-H), 1.5-1.8 (4 H, m, 8-H, 9-H, 10-H), 1.9 (1 H, m, 19-H), 2.0-2.2 (4 H, m, 11-H, 20-H), 2.5 (1 H, m, 19-H), 2.7 (1 H, d, *J* 12.8, 1-H), 2.8 (3 H, m, 1-H, 21-H), 3.0 (1 H, m, 18-H), 3.1 (2 H, m, 17-H), 3.3 (1 H, m, 7-H), 3.5 (1 H, m, 17-H), 4.2 (1 H, dd, *J* 8.0 and 4.8, 6-H), 4.4 (1 H, m, 2-H), 4.9 (1 H, dd, *J* 8.0 and 7.6, 14-H),

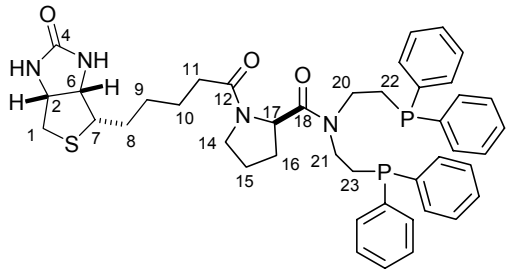
6.9 (2 H, m, 23-H, 27-H), 7.1 (3 H, m, 24-H, 25-H, 26-H), 7.3-7.4 (20 H, m, Ph) and 8.0 (1 H, d, *J* 8.0, 13-H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 26.0 (10-C), 26.6 (20-C), 27.7 (19-C), 27.8 (8-C), 28.1 (9-C), 35.6 (11-C), 39.0 (21-C), 41.0 (1-C), 44.6 (18-C), 45.6 (17-C), 50.5 (14-C), 56.0 (7-C), 60.8 (2-C), 62.0 (6-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 165.2 (4-C), 172.8 (15-C) and 173.8 (12-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -20.1 and -20.3; m/z (ESI) 837.5 (M^+ + Na, 100%) 869.5 (10), 853.3 (18).

Procedure for the Synthesis of (*S*)-*N*,*N*-bis(2-(diphenylphosphino)ethyl)-1-(5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl)pyrrolidine-2-carboxamide: Biot-(*S*)-Pro-1



N-*N*-Diisopropylethylamine (0.11 ml, 0.68 mmol) was added to a solution containing biotin pentafluorophenyl ester (0.07 g, 0.17 mmol) and the deprotected amino acid diphosphine H-(*S*)-Pro-1 (0.35 mmol) in dimethylformamide (10 ml). The mixture was stirred 48 h at room temperature. The solvent was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using chloroform / ethanol (8/2) as eluent, allowing isolation of pure product **Biot-(*S*)-Pro-1** (0.07 g, 54%) as a white foam. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.4-1.5 (2 H, m, 10-H, 9-H), 1.6-1.8 (7 H, m, 8-H, 15-H, 16-H, 10-H, 9-H), 2.1 (1 H, m, 15-H), 2.2-2.4 (5 H, m, 22-H, 23-H, 11-H), 2.5 (1 H, m, 23-H), 2.7 (1 H, d, *J* 12.8, 1-H), 2.8 (1 H, dd, *J* 12.8 and 4.8, 1-H), 3.1 (1 H, m, 7-H), 3.2-3.4 (3 H, m, 20-H, 21-H), 3.5 (1 H, m, 14-H), 3.5-3.7 (2 H, m, 21-H, 14-H), 4.2 (2 H, m, 6-H, 17-H), 4.4 (1 H, m, 2-H), 5.4 (1 H, br, biotin NH), 6.3 (1 H, br, biotin NH) and 7.3-7.4 (20 H, m, Ph); δ_C(100 MHz; $CDCl_3$; Me_4Si) 24.9 (15-C), 26.8 (23-C), 27.0(22-C), 27.9 (8-C), 28.5 (9-C), 28.8 (10-C), 29.4 (16-C), 33.9 (11-C), 40.9 (1-C), 44.6 (20-C), 45.5 (21-C), 48.0 (14-C), 56.0 (7-C), 57.1 (17-C), 60.6 (2-C), 62.1 (6-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 164.1 (4-C), 171.9 (18-C) and 172.0 (12-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -19.7 and -20.5; m/z (ESI) 787.5 (M^+ + Na, 100%), 819.3 (20), 803.4 (35).

Procedure for the Synthesis of (*R*)-*N*,*N*-bis(2-(diphenylphosphino)ethyl)-1-(5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl)pyrrolidine-2-carboxamide: Biot-(*R*)-Pro-1



N-*N*-Diisopropylethylamine (0.44 ml, 2.68 mmol) was added to a solution containing biotin pentafluorophenyl ester (0.28 g, 0.67 mmol) and the deprotected amino acid diphosphine H-(*R*)-Pro-1 (0.67 mmol) in dimethylformamide (10 ml). The mixture was stirred 48 h at room temperature. The solvent was removed *in vacuo* and the crude precipitate was purified by column chromatography on silica gel 60 F254 using chloroform / ethanol (8/2) as eluent, allowing isolation of pure product **Biot-(*R*)-Pro-1** (0.29 g, 58%) as a white foam. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.3-1.4 (2 H, m, 10-H, 9-H), 1.6-1.8 (7 H, m, 8-H, 15-H, 16-H, 10-H, 9-H), 2.0 (1 H, m, 15-H), 2.1-2.3 (4 H, m, 22-H, 23-H, 11-H), 2.4-2.6 (4 H, m, 23-H, 1-H, 11-H), 3.0 (1 H, m, 7-H), 3.1 (1 H, m, 20-H), 3.3 (2 H, m, 21-H), 3.4 (1 H, m, 14-H), 3.6 (1 H, m, 20-H), 3.8 (1 H, m, 14-H), 4.2 (2 H, m, 6-H, 2-H), 4.4 (1 H, m, 17-H), 5.4 (1 H, br, biotin NH), 6.3 (1 H, br, biotin NH) and 7.3-7.4 (20 H, m, Ph); δ_C(100 MHz; $CDCl_3$; Me_4Si) 24.9 (15-C), 26.8 (23-C), 27.0 (22-C), 27.9 (8-C), 28.5 (9-C), 28.8 (10-C), 29.4 (16-C), 33.9 (11-C), 40.9 (1-C), 44.6 (20-C), 45.5 (21-C), 48.0 (14-C), 56.0 (7-C), 57.1 (17-C), 60.6 (2-C), 62.1 (6-C), 128.8-133.6 (Ph–H), 136.9-138.2 (Ph–C), 164.1 (4-C), 171.9 (18-C) and 172.0 (12-C); δ_P(162 MHz; $CDCl_3$; H_3PO_4) -19.8 and -20.4; m/z (ESI) 765.3 (M^+ + H, 100%), 781.3 (10).

Hydrogenation protocol

Considering the very small amounts of catalyst involved, we found that the reproducibility of the results critically depends on the care taken in the preparation of the catalytic runs. All organic and aqueous solutions were deoxygenated by flushing nitrogen through the solutions for at least three hours. All operations were performed in a glove box. The following buffers were screened: MES: 2-(*N*-morpholino)ethanesulfonic acid sodium salt, pH 5.5, 0.1 M final

conc. for experiments using streptavidine, and MOPS: 3-(*N*-morpholino)propanesulfonic acid sodium salt pH 7.0, 0.1 M final conc. for experiments using avidine.

The metal source $[Rh(COD)_2]BF_4$ (4 mg, 10 μmol) was dissolved in DMSO and 1.56 μmol of this solution was added to the appropriate aliquoted ligand (2.02 μmol, 1.3 equivalents with respect to the metal source). The solution was stirred at rt for 15 min. This solution can be stored and reused for a few days without any noticeable loss of activity and of selectivity.

Homogenous hydrogenation:

A Pyrex tube (volume ca. 3 mL) was placed in an autoclave and charged with an *N*-acetamidoacrylic acid solution in buffer (MOPS or MES) 0.38M (130 μl of a 23.85 mM solution, 3.10 μmol), and an *N*-acetamidocinnamic acid solution in buffer (MOPS or MES) 0.38M (130 μl of a 23.85 mM solution, 3.10 μmol). The volume was adjusted with water to 900 μl. The protein solution in water was added (100 μl of a 0.207 mM solution, 0.0207 μmol of the tetramer). The precatalyst solution in DMSO (100 μl, 0.062 μmol) was added last. The resulting mixture was vortexed and hydrogenated at 5 bar.

Bi-phasic hydrogenation:

In an autoclave (volume ca. 10 mL) an *N*-acetamidoacrylic acid solution in ethyl acetate (550 μl of a 56.59 mM solution, 3.10 μmol), and an *N*-acetamidocinnamic acid solution in ethyl acetate (550 μl of a 56.59 mM solution, 3.10 μmol) were charged. The buffer solution (580 μL of a 0.38 M solution) was added and the volume of the aqueous phase adjusted with water to 975 μl. The protein solution in water was then added (100 μl of a 0.207 mM solution, 0.0207 μmol of the tetramer). The precatalyst solution in DMSO (25 μl, 0.062 μmol) was added last. The resulting mixture was vortexed and hydrogenated at 5 bar.

The glass autoclave containing up to twenty one samples was closed, purged under vacuum and charged with H_2.

After stirring for 15 hrs on an orbital shaker at the desired temperature, the reaction was quenched by adjusting the pH to 2 with 2.0 N aqueous HCl solution. The aqueous solution was continuously extracted with ethyl acetate for 2 hrs and the organic phase was evaporated to a minimum volume (0.5 mL). The enantiomeric excess (ee) was determined on chiral columns : Permabond-L-Chirasyl-Val (Macheray-Nagel), 25 m x 0.25 mm column: He carrier gas: 1.2 bar, inlet injector 280°C, oven (85°C; 10 min, 8°C/min; 135°C; 0 min, 10°C/min;

180°C; 35min), FID detector 200 °C; retention time: Methyl *N*-acetylacetamidoacrylate: 5.8 min, Methyl (*R*)-*N*-acetylacetamidoalanine: 11.3 min, Methyl (*S*)-*N*-acetylacetamidoalanine: 12.8 min, Methyl (*R*)-*N*-acetylacetamidophenylalanine: 24.1 min, Methyl (*S*)-*N*-acetylacetamidophenylalanine: 24.7 min, Methyl *N*-acetylacetamidocinnamate: 34.2 min.