Journal of Organometallic Chemistry 694 (2009) 930–936

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Incorporation of biotinylated manganese-salen complexes into streptavidin: New artificial metalloenzymes for enantioselective sulfoxidation

A. Pordea [a], D. Mathis [a], T.R. Ward [a,b,*]

[a] Institute of Chemistry, University of Neuchâtel, Avenue Bellevaux 51, CP 158, CH-2009 Neuchâtel, Switzerland
[b] Department of Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

ARTICLE INFO

Article history:
Received 17 September 2008
Received in revised form 10 November 2008
Accepted 11 November 2008
Available online 19 November 2008

This work is dedicated to Prof. G. Jaouen on the occasion of his 65th birthday.

Keywords:
Enantioselective catalysis
Artificial metalloenzyme
Biotin–avidin technology
Oxidation
Schiff base

ABSTRACT

Incorporation of achiral biotinylated manganese-salen complexes into streptavidin yields artificial metalloenzymes for aqueous sulfoxidation using hydrogen peroxide. Four biotinylated salen ligands were synthesized and their manganese complexes were tested in combination with several streptavidin mutants, yielding moderate conversions (up to 56%) and low enantioselectivities (maximum of 13% ee) for the sulfoxidation of thioanisole.

1. Introduction

The steric and electronic properties of organometallic complexes synthesized by chemists enable them to catalyze many chemical transformations. Their application in asymmetric catalysis is one of the most efficient routes to enantiopure compounds. In nature, it is estimated that half of all enzymes contain a metal ion [1]. The complex structures and functions of such metalloenzymes have inspired chemists and biochemists in designing highly efficient and enantioselective catalytic systems. However, the highly organized protein framework around the metal center provides very precise second coordination sphere interactions, which are difficult to mimic in traditional organometallic chemistry. The design of artificial metalloenzymes for asymmetric catalysis is based on combining the high activity of an organometallic complex with the enantioselectivity provided by the protein environment.

Several strategies can be envisaged for the incorporation of a catalytically active metal center inside a protein. Supramolecular, dative or covalent anchoring have been successfully implemented for the creation of artificial metalloenzymes that catalyze different enantioselective transformations including: ester hydrolysis [2], reductions (hydrogenation, transfer hydrogenation) [3–14], oxidations (dihydroxylation, epoxidation, sulfoxidation) [15–23] or C–C bond formations (Diels–Alder, Michael additions and allylic alkylation) [24,25]. In this context, we have demonstrated that the biotin-(strept)avidin technology, first reported by Whitesides et al. [3], offers a great potential for the design and optimization of hydrogenation [7], transfer hydrogenation [13,14], alcohol oxidation [26] and allylic alkylation reactions [25]. With the aim of expanding the reaction range of this type of hybrid catalysts, we focused our attention on developing enantioselective oxidations and we selected the sulfoxidation reaction as a starting point.

Among the metal catalysts for asymmetric oxidations, salen-based complexes have received considerable attention, due to their synthetic accessibility, versatility and high catalytic activity [27–29]. Highly enantioselective aqueous oxidation systems based on salen, salan or salalen complexes of Ti, Fe and Al have been reported recently [30–33]. Although their application in homogeneous asymmetric sulfoxidation is limited [34–36], manganese- and chromium-salen derivatives are water compatible and have already been used in the context of artificial metalloenzymes as mimics of heme, the native co-factor of myoglobin [20,21]. Herein, we describe the creation of streptavidin-based artificial metalloenzymes for sulfoxidation reactions using achiral biotinylated Mn-salen complexes. The proposed mechanism of the oxidation

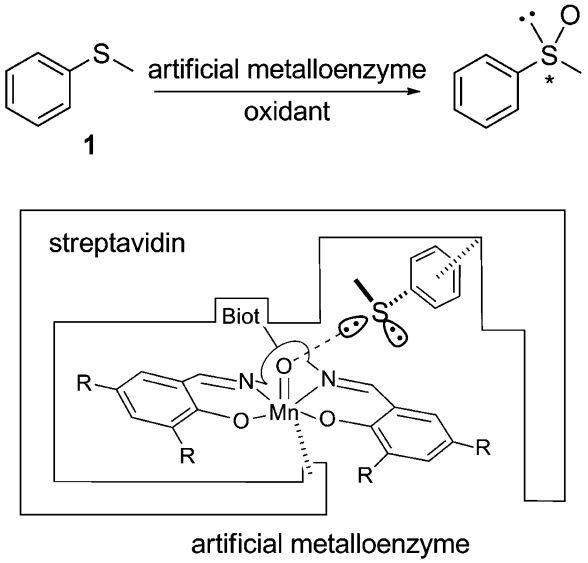
* Corresponding author. Address: Department of Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland. Tel.: +41 61 267 1004; fax: +41 61 267 1005.
E-mail address: thomas.ward@unibas.ch (T.R. Ward).

0022-328X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2008.11.023

reactions catalyzed by Mn- or Cr-salens, which does not require binding of the substrate to the metal [37,38], suggests that the chiral environment provided by the protein could have a determinant influence on the enantiodiscrimination step. Cationic achiral Mn-salen complexes have previously been used as chiral catalysts in the presence of an optically active donor axial ligand, which favors one particular conformation of the five-membered chelate ring formed between the manganese ion and the ethylenediimine part [39]. In this context, we reasoned that the host protein could influence either delivery of the incoming prochiral substrate, or the conformation of the organometallic moiety, to provide enantioselectivity (Scheme 1).

2. Results and discussion

Metal-salen complexes have already been used by the groups of Watanabe and Lu for the creation of artificial metalloenzymes for asymmetric sulfoxidation, by incorporation into apo-myoglobin. In one case, the binding affinity of apo-myoglobin for manganese- and chromium-salen complexes was ensured by hydrophobic interactions, as well as by a dative bond between the metal and a hystidine residue. The rational design based on structural information allowed chemical and genetic fine-tuning of the metal position inside the protein and thus, optimization of the binding and of the enantioselectivity (up to 33% (*S*) for the sulfoxidation of thioanisole) [19,20]. On the other hand, Lu and coworkers have used a dual covalent anchoring of a manganese-salen into apo-myoglobin, thus restraining the flexibility of the metal and improving enantioselectivity (up to 51% (*S*) for the sulfoxidation of thioanisole) [21]. In this context, and inspired by Jacobsen's catalyst [40], one of the most successful metal-salen complexes for the epoxidation of alkenes, we designed and synthesized the achiral biotinylated salen ligand **Sal-1**, in which the diimine bridge was derived from a sterically constrained *cis*-3,4-diaminopyrrolidine (Scheme 2). The diamine precursor was synthesized in five steps starting from *N*-benzylmaleimide by ruthenium-catalyzed dihydroxylation, reduction of the dihydroxyimide and ditosylation of the corresponding diol, followed by conversion to the diazidopyrrolidine, which was reduced to the diamine with simultaneous deprotection of the benzyl group by hydrogenation in presence of Pd/C. Condensation with 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde and biotinylation of the corresponding diiminopyrrolidine yielded the biotinylated salen ligand **Sal-1** (see the Section 4 for the description of the synthetic procedures and the characterization of the intermediates). The manganese-salen complex, Mn-**Sal-1**, was prepared by treatment with manganese acetate, $Mn(OAc)_2 \cdot 4H_2O$, followed by ligand exchange with NaCl (replacement of acetate by the chloride ion) [41] and purification on silica gel.



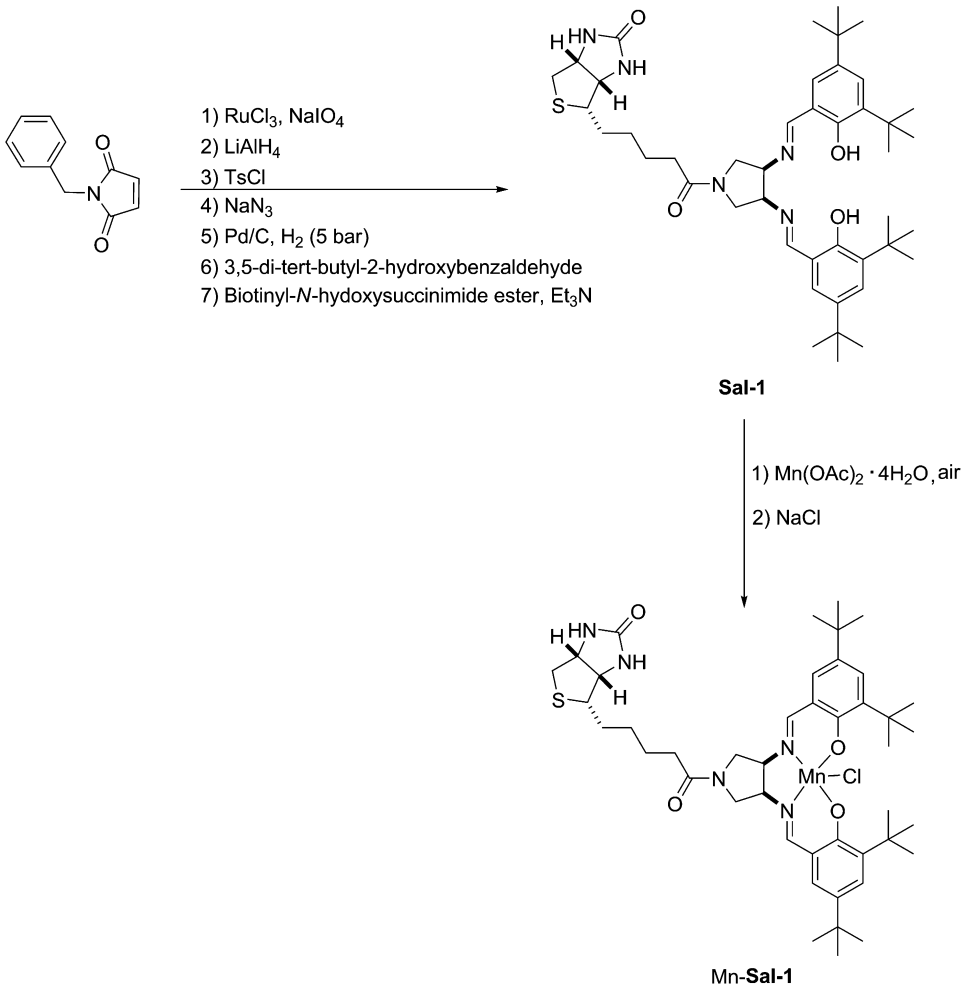
Scheme 1. Schematic representation of possible interactions during the sulfoxidation of thioanisole catalyzed by biotinylated manganese-salen complexes incorporated into streptavidin.

Among the variety of oxidants used in combination with Mn-salen complexes for catalytic oxidations, we selected hydrogen peroxide for initial sulfoxidation experiments, because of its water compatibility and due to a low extent of background reaction (uncatalyzed oxidation leading to racemic sulfoxide, Table 1, entry 1). In combination with H_2O_2, Mn-**Sal-1** displayed a very modest activity for the sulfoxidation of thioanisole **1** (Table 1, entry 2). As the complex was only poorly soluble in water, the experiment was repeated in a H_2O/EtOH 1/1 mixture (v/v), thus affording a reasonable conversion (Table 1, entry 3). Reasoning that incorporation into streptavidin would solve the solubility problem, we proceeded to test the catalyst in the presence of the host protein. Upon adding WT Sav (hereafter, Sav refers to streptavidin; wild-type streptavidin is abbreviated WT Sav) to the reaction mixture, a slight increase in conversion was observed, but the reaction displayed little selectivity (Table 1, entry 4).

Two approaches can be envisaged for the optimization of such artificial metalloenzymes: genetic modification of the host protein or chemical modifications of the first coordination sphere of the metal. During this study, in the absence of precise information on the localization of the metal center inside streptavidin, the L7,8 loop region (residues 112–121) was selected for the genetic optimization, as it lied close to the carboxylate of biotin's side chain and therefore could perturb the environment around the organometallic fragment. In a first attempt to optimize the activities and selectivities of Mn-**Sal-1** $\subset$ Sav ($\subset$ refers to the incorporation of the biotinylated complex into Sav), we tested the influence of point mutations at the 112 position, expected to lie in the proximity of the organometallic moiety. A small increase in activity was observed upon using the S112G Sav mutant (Table 1, entry 5). Interestingly, S112D and S112G mutations afforded a reversed selectivity compared to WT Sav, suggesting that the host protein has an influence on the enantioselection (Table 1, entries 5–6).

Previous results obtained for hydrogenation [7], transfer hydrogenation [13] and allylic alkylation reactions [25] demonstrated that chemical optimization brings more diversity than genetic modifications. Therefore, in a second approach, three additional biotinylated Schiff base tetradentate ligands were synthesized and their Mn complexes, formed *in situ*, were tested in the sulfoxidation of thioanisole.

To maximize the possible interactions upon incorporation into streptavidin, the biotinylated ligands were designed to offer a large structural diversity, by varying the ligand type (aromatic or aliphatic imines), the biotin anchor position (on the diimine backbone or on the 2-hydroxybenzaldehyde moiety), and the spacer between the biotin and the coordinating moiety. The three achiral salen ligands **Sal-2**, **Sal-3** and **Sal-4** (Scheme 3) were synthesized by condensation of the appropriate diamine with the corresponding 2-hydroxybenzaldehydes. 3,5-Di-*tert*-butyl-2-hydroxybenzaldehyde was used to form the symmetric diimines **Sal-2** and **Sal-4**, and the biotin anchor was attached to the diamine component before (**Sal-2**) or after Schiff base formation (**Sal-4**). The non-symmetric diimine **Sal-3** was obtained upon mixing ethylenediamine, 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde and the aldehyde bearing the biotin anchor (see Section 4).



Scheme 2. Synthesis of the biotinylated manganese complex Mn-**Sal-1**.

Table 1
Sulfoxidation of thioanisole catalyzed by Mn-**Sal-1**.[a]

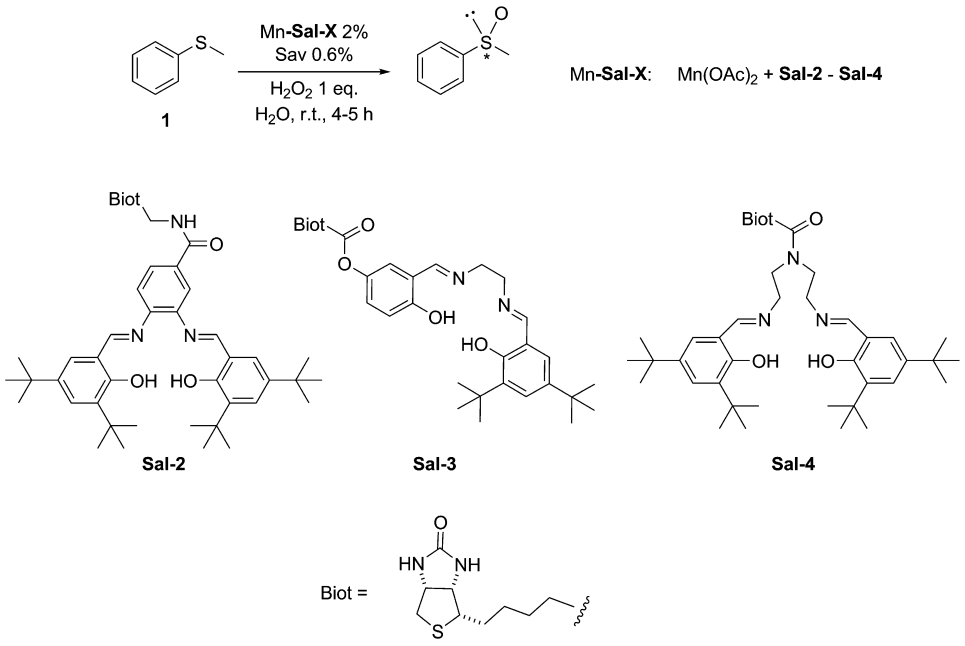
Entry	Protein	Catalyst	Solvent	Conv. (%)	ee (%)
1	–	–	H_2O	10	–
2	–	Mn-**Sal-1**	H_2O	10	–
3	–	Mn-**Sal-1**	H_2O/EtOH 1/1	50	–
4	WT Sav	Mn-**Sal-1**	H_2O	21	6 (*R*)
5	S112G Sav	Mn-**Sal-1**	H_2O	34	11 (*S*)
6	S112D Sav	Mn-**Sal-1**	H_2O	25	5 (*S*)

[a] *Reaction conditions*: 1.5% DMF in H_2O; Sav 60 μM (tetrameric); Mn-**Sal-1** 200 μM; thioanisole **1** 10 mM; H_2O_2 1 equiv.; room temperature; 4 h.

The new biotinylated Mn-salen complexes catalyzed the aqueous sulfoxidation of thioanisole with modest activities in the presence of H_2O_2 as stoichiometric oxidant (Table 2, entries 1–3). A slight decrease in conversion was obtained with all the catalysts in the presence of S112D Sav (Table 2, entries 4–6) and the enantioselectivities remained low (maximum 13% ee (*R*) for Mn-**Sal-3** ⊂ S112D Sav, Table 2, entry 5). It is worthy to note that replacing the **Sal-1** ligand with the non-symmetric diimine **Sal-3** lead to an inversion of the enantioselectivity in the presence of the S112D Sav mutant (from 5% ee (*S*) – Table 1, entry 6, to 13% ee (*R*) – Table 2, entry 5). Several streptavidin isoforms bearing glycine or alanine mutations in the L7,8 loop region were then tested for enantioselective sulfoxidation after incorporation of the Mn-**Sal-3** catalyst (Table 2, entries 7–9). Interestingly, the T115A and E116A Sav mutants afforded a considerably increased activity (Table 2, entries 8–9). Finally, the addition of coordinating axial ligands such as *N*-methylimidazole or pyridine-*N*-oxide, known to be beneficial for the Mn-catalyzed reactions with H_2O_2 [42,43], did not improve the activity or the selectivity of the catalysts (Table 2, entries 10–11).

3. Conclusion

In summary, we showed that incorporation of achiral biotinylated manganese-salen complexes into streptavidin yields active artificial metalloenzymes for the sulfoxidation of thioanisole. Although the enantioselectivities obtained are low compared to other strategies based on the association of metal-salen complexes with proteins [19–21], they suggest that the protein participates in the enantioselection mechanism. Moreover, the tight binding being ensured by the biotin anchor, a high diversity might be introduced by chemical derivatization without noticeable loss in affinity. The structures of the organometallic salen-type catalysts can be readily tuned [27] to provide a variety of biotinylated ligands, which can be combined with genetic variations of the host protein, in order to reduce the flexibility of the metallic moiety and thus increase enantioselectivity. In contrast to methods relying on the incorporation of an isolated metal ion into a protein [15–18,23], this method overcomes the problem of affinity between the two counterparts and offers the advantage of a well-defined binding site for the catalytic center. Ongoing research is directed towards computer mod-



Scheme 3. Biotinylated salen ligands **Sal-2**, **Sal-3** and **Sal-4**, tested for the sulfoxidation of thioanisole **1**.

Table 2
Sulfoxidation of thioanisole catalyzed by Mn-**Sal-2**–Mn-**Sal-4**.[a]

Entry	Protein	Catalyst	Additive	Conv. (%)	ee (%)
1	–	Mn-**Sal-2**	–	44	–
2	–	Mn-**Sal-3**	–	38	–
3	–	Mn-**Sal-4**	–	19	–
4	S112D Sav	Mn-**Sal-2**	–	30	1 (*S*)
5	S112D Sav	Mn-**Sal-3**	–	32	13 (*R*)
6	S112D Sav	Mn-**Sal-4**	–	3	0
7	T114G Sav	Mn-**Sal-3**	–	32	8 (*R*)
8	T115A Sav	Mn-**Sal-3**	–	56	6 (*R*)
9	E116A Sav	Mn-**Sal-3**	–	56	7 (*R*)
10	S112D Sav	Mn-**Sal-3**	Pyridine-*N*-oxide	36	13 (*R*)
11	S112D Sav	Mn-**Sal-3**	*N*-Methylimidazole	35	11 (*R*)

[a] *Reaction conditions*: 1% DMF in H_2O; Sav 33 μM (tetrameric); Mn-**Sal-X** 100 μM; thioanisole **1** 5 mM; H_2O_2 1 equiv.; room temperature; 5 h.

eling and structural characterization, which would allow gaining more information on the localization of the active site and thus optimization of the activity and of the selectivity of these metalloenzymes.

4. Experimental

4.1. General considerations

Solvents were of analytical grade and were purchased from Aldrich, Fluka or Acros and used without further purification. Water was purified to milliQ degree of purity. D-Biotin was purchased from Chanzou Huaren (purity ⩾ 99%). Biotin pentafluorophenol ester (BPFP) and biotinyl-*N*-hydroxysuccinimide ester (BNHS) were prepared according to reported procedures [44,45]. ^1H and ^{13}C were acquired on a 200 MHz (Varian) or on a 400 MHz (Bruker) spectrometer using deuterated chloroform (99.8% D), dimethyl sulfoxide (99.5% D) or methanol (99.8% D) from Cambridge Isotope Laboratories. Chemical shifts are reported in ppm and signals are quoted as s (singulet), d (doublet), t (triplet), br (broad) or m (multiplet). Electron Spray Ionization and Atmospheric Pressure Chemical mass spectra were recorded on a LCQ-IT (Finnigan) spectrometer. The relative intensities are given in parenthesis (%). Streptavidin (wild-type and mutants) was prepared according to published results [46].

4.2. Synthesis of (meso)-N-biotinyl-3,4-(N′,N″-bis(3,5-di-tert-butylsalicylidene))-pyrrolidine, *Sal-1*

4.2.1. (meso)-N-Benzyl-3,4-dihydroxy-2,5-pyrrolidinedione [48]

To a solution of *N*-benzylmaleimide (3 g, 16.0 mmol) in AcOEt/CH_3CN 1/1 (total volume 195 mL) cooled in an ice bath at 0 °C was added a solution of $RuCl_3$ (233 mg, 1.13 mmol) and $NaIO_4$ in water (32 mL). The biphasic mixture was vigourously stirred for 3 min at 0 °C, following which the reaction was quenched with 100 mL $Na_2S_2O_3$ saturated solution. The organic phase was separated and the aqueous layer was extracted three times with AcOEt. The combined organic layers were dried over $MgSO_4$ and evaporated *in vacuo* to afford the crude product, which was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (98/2) to afford a white solid (2.5 g, 70% yield). ^1H NMR (200 MHz, CD_3OD, 298 K): δ_H (ppm) 4.48 (s, 2H, N-CH_2-Ph), 4.67 (s, 2H, 2 × C*H*-OH), 7.28–7.34 (m, 5H, $HC_{aromatic}$).

4.2.2. (meso)-N-Benzyl-3,4-dihydroxypyrrolidine

This synthesis was adapted from a published procedure [48]. All the manipulations were carried out using standard Schlenk techniques. To a suspension of $LiAlH_4$ (4.3 g, 113 mmol) in THF (130 mL) under nitrogen was added a solution of (*meso*)-*N*-benzyl-3,4-dihydroxy-2,5-pyrrolidinedione (5.0 g, 22.6 mmol) and the reaction mixture was refluxed during 62 h. The reaction was cooled with an ice bath and AcOEt (120 mL) was carefully added with vigourous stirring, followed by water (18 mL) and 3 M NaOH solution (4.25 mL). After 1 h stirring at room temperature, the aluminium salts filtered on celite and washed with AcOEt. The filtrate was evaporated to yield the crude product, which was purified by flash chromatography on silicagel using AcOEt/MeOH/Et_3N (95/5/1) to afford a yellow oil (1.13 g, 26% yield). ^1H NMR (200 MHz, $CDCl_3$, 298 K): δ_H (ppm) 2.59 (br, 2H, O*H*), 2.68–2.70 (m, 4H, 2 × CH_2-N), 3.6 (s, 2H, N-CH_2-Ph), 4.16–4.21 (m, 2H, 2 × C*H*-OH), 7.28–7.37 (m, 5H, $HC_{aromatic}$).

4.2.3. (meso)-N-Benzyl-3,4-dihydroxypyrrolidine di-p-toluenesulfonate

This synthesis was adapted from a published procedure [49]. To a solution of (*meso*)-*N*-benzyl-3,4-dihydroxypyrrolidine (1.13 g, 5.85 mmol) in pyridine (32 mL) was added *p*-toluenesulfonyl chloride (3.4 g, 17.8 mmol) in one portion. The resulting solution was stored at 4 °C for 4 days. The mixture was then added dropwise to a cooled solution of citric acid (160 g in 1 L water) and the resulting aqueous solution was extracted four times with Et_2O. The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash chromatography on silicagel using CH_2Cl_2/Et_3N (100/1) to afford a white solid (2.36 g, 81% yield). ^{1}H NMR (200 MHz, $CDCl_3$, 298 K): δ_H (ppm) 2.47 (s, 6H, 2 × C*H*$_3$), 2.69–2.76 (m, 2H, C*H*$_2$-N), 3.02–3.11 (m, 2H, C*H*$_2$-N), 3.6 (s, 2H, N-C*H*$_2$-Ph), 4.71–4.83 (m, 2H, 2 × C*H*-OH), 7.19–7.36 (m, 9H, *H*C$_{aromatic}$), 7.74 (s, 2H, *H*C$_{aromatic}$), 7.78 (s, 2H, *H*C$_{aromatic}$).

4.2.4. (meso)-N-Benzyl-3,4-diazidopyrrolidine

This synthesis was adapted from a published procedure [50]. All the manipulations were carried out using standard Schlenk techniques. To a solution of (*meso*)-*N*-benzyl-3,4-dihydroxypyrrolidine di-*p*-toluenesulfonate (2.0 g, 4.0 mmol) in DMF (22 mL) under nitrogen was added solid NaN_3 (1.04 g, 16.0 mmol). The resulting suspension was heated at 100 °C for 22 h and then cooled at room temperature. Water (40 mL) was added and the mixture was extracted four times with CH_2Cl_2. The organic layers were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash chromatography on silicagel using hexane/AcOEt (80/20) to afford an incolor oil (880 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ_H (ppm) 2.70 (dd, *J* = 10.2 Hz, *J* = 4.7 Hz, 2H, 2 × C*H*$_2$-N), 3.01 (dd, *J* = 10.2 Hz, *J* = 6.5 Hz, 2H, 2 × C*H*$_2$-N), 3.69 (s, 2H, N-C*H*$_2$-Ph), 4.04–4.07 (m, 2H, 2 × C*H*-N$_3$), 7.32–7.38 (m, 5H, *H*C$_{aromatic}$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ_C (ppm) 57.5 (2 × *C*H$_2$-N), 60.1 (N-*C*H$_2$-Ph), 62.2 (2 × *C*H-N$_3$), 127.8 (*C*$_{aromatic}$H), 128.9 (2 × *C*$_{aromatic}$H), 129.1 (2 × *C*$_{aromatic}$H), 138.2 (*C*$_{aromatic}$).

4.2.5. (meso)-3,4-(N,N′-Bis(3,5-di-tert-butylsalicylidene))-pyrrolidine

This synthesis was adapted from a published procedure [51]. All the manipulations were carried out using standard Schlenk techniques. A suspension of palladium on charcoal (10% w/w, 47 mg, 0.02 mmol) in EtOH/CF_3COOH (8/2 v/v) was added to a solution of (*meso*)-*N*-benzyl-3,4-diazidopyrrolidine (100 mg, 0.41 mmol) in EtOH/CF_3COOH (8/2 v/v, total volume 2 mL). The suspension was shaken under 5 bar H_2 for 16 h, filtered through celite and concentrated under vacuum to afford a white solid. The crude residue was dissolved in EtOH (3 mL) and neutralized with a solution of NaOH 4.7 M (0.32 mL). Neat 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (197 mg, 0.84 mmol) was added at once and the reaction was refluxed for 2 h. The reaction mixture was then poured into 3.3 mL saturated NaCl solution and extracted three times with CH_2Cl_2. The organic layers were combined, washed with saturated NaCl solution, dried over Na_2SO_4 and evaporated under vacuum. The product was purified by flash chromatography on silicagel using AcOEt/MeOH (initially pure AcOEt, then 95/5) to yield a foamy yellow glass (110 mg, 54% yield over the two steps). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ_H (ppm) 1.28 (s, 18H, 2 × C(C*H*$_3$)$_3$), 1.29 (s, 18H, 2 × C(C*H*$_3$)$_3$), 3.39–3.41 (m, 4H, 2 × C*H*$_2$-N), 4.05–4.07 (m, 2H, C*H*-N=CH), 7.04 (d, *J* = 2.44 Hz, 2H, *H*C$_{aromatic}$), 7.35 (d, *J* = 2.44 Hz, 2H, *H*C$_{aromatic}$), 8.36 (s, 2H, 2 × C*H*=N), 13.34 (s, 2H, 2 × O*H*). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ_C (ppm) 29.8 (C-(*C*H$_3$)$_3$), 31.9 (C-(*C*H$_3$)$_3$), 34.5 (2 × *C*-CH$_3$), 35.4 (2 × *C*-CH$_3$), 53.4 (2 × *C*H$_2$-N), 73.6 (2 × *C*H-N), 118.0 (2 × *C*$_{aromatic}$), 126.2 (2 × *C*$_{aromatic}$H), 127.5 (2 × *C*$_{aromatic}$H), 137.1 (2 × *C*$_{aromatic}$), 140.2 (2 × *C*$_{aromatic}$), 158.5 (*C*$_{aromatic}$), 166.7 (2 × *C*H=N). APCI$^+$-MS: 546.5 (30), 535.5 (50, [M+2H]$^{2+}$), 534.5 (100, [M+H]$^+$).

4.2.6. (meso)-N-Biotinyl-3,4-(N′,N″-bis(3,5-di-tert-butylsalicylidene))-pyrrolidine, ***Sal-1***

To a solution of (*meso*)-3,4-(*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene))-pyrrolidine (36 mg, 0.07 mmol) and BNHS (25 mg, 0.07 mmol) in DMF (1 mL) was added Et_3N (14 μL, 0.1 mmol) and the reaction mixture was stirred at room temperature during 24 h. The solvent was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (90/10) to afford **Sal-1** as a yellow solid (45 mg, 86% yield).

^{1}H NMR (200 MHz, $CDCl_3$, 298 K): δ_H (ppm) 1.27–1.37 (m, 36H, 4 × C(C*H*$_3$)$_3$), 1.40–1.90 (m, 6H, C*H*$_2$C*H*$_2$C*H*$_2$-CH$_2$CO), 2.41 (m, 2H, C*H*$_2$-CO), 2.6–3.0 (m, 2H, C*H*$_2$-S), 3.11–3.25 (m, 1H, C*H*-S), 3.8–3.97 (m, 4H, 2 × C*H*$_2$-N), 4.0–4.25 (m, 2H, 2 × C*H*-N=CH), 4.30–4.40 (m, 1H, C*H*-N), 4.45–4.50 (m, 1H, C*H*-N), 5.30 (d, *J* = 5.6 Hz, 1H, CH-N*H*), 5.97 (d, *J* = 9.6 Hz 2H, CH-N*H*), 7.05 (dd, *J* = 2.4 Hz, *J* = 4.4 Hz, 2H, 2 × *H*C$_{aromatic}$), 7.36 (d, *J* = 2.4 Hz, 2H, 2 × *H*C$_{aromatic}$), 8.35 (s, 1H, C*H*=N), 8.42 (s, 1H, C*H*=N), 13.0 (s, 1H, O*H*), 13.07 (s, 1H, O*H*).

ESI$^-$-MS: 758.7 (100, [M−H]$^-$), 759.7 (50, M), 794.5 (80, [M−H+2H$_2$O]$^-$), 795.4 (40, M+2H$_2$O).

4.3. Synthesis of N,N′-bis-(3,5-di-tert-butylsalicylidene)-3,4-diaminobenzoic acid, aminobiotinyl amide, ***Sal-2***

4.3.1. 3,4-Diaminobenzoic acid, aminobiotinyl amide

Biotinamine was synthesized according to a reported procedure [44]. 3,4-Diaminobenzoic acid (152 mg, 1 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide EDC (233 mg, 1.5 mmol), 1-hydroxybenzotriazole HOBT (202 mg, 1.5 mmol), Et_3N (268 μL, 2 mmol) and biotinamine (401 mg, 1.75 mmol) were mixed in DMF (40 mL) and the resulting solution was stirred at 45 °C during 24 h. After this time, the DMF was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (initial mixture 9/1, final mixture 6/4) to afford the product (203 mg, 0.56 mmol, 56% yield).

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) 1.21–1.61 (m, 8H, C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$-CH$_2$NH), 2.58 (d, *J* = 12.4 Hz, 1H, C*H*$_2$-S), 2.82 (dd, *J* = 12.4 Hz, *J* = 5.1 Hz, 1H, C*H*$_2$-S), 3.08–3.14 (m, 1H, C*H*-S), 3.38–3.52 (m, 2H, C*H*$_2$-NH), 4.12–4.15 (m, 1H, C*H*-N), 4.29–4.33 (m, 1H, C*H*-N), 4.95 (br, 4H, N*H*$_2$), 6.39 (s, 1H, CH-N*H*), 6.47 (s, 1H, CH-N*H*), 6.47 (d, *J* = 8.0 Hz, 1H, *H*C$_{aromatic}$), 6.95 (dd, *J* = 8.0 Hz, *J* = 1.9 Hz, 1H, *H*C$_{aromatic}$), 7.04 (d, *J* = 1.9 Hz, 1H, *H*C$_{aromatic}$), 7.88 (t, *J* = 5.6 Hz, 1H, CH$_2$-*H*N).

^{13}C NMR (100 MHz, DMSO-d_6, 298 K): δ_C (ppm) 27.4 (*C*H$_2$), 29.1 (*C*H$_2$), 29.2 (*C*H$_2$), 30.1 (*C*H$_2$), 39.7 (*C*H$_2$-NH), 40.7 (*C*H$_2$-S), 56.4 (*C*H-S), 60.1 (*C*H-N), 61.9 (*C*H-N), 113.6 (*C*$_{aromatic}$H), 114.7 (*C*$_{aromatic}$H), 117.8 (*C*$_{aromatic}$H), 124.1 (*C*$_{aromatic}$-CO), 134.7 (*C*$_{aromatic}$-NH$_2$), 139.0 (*C*$_{aromatic}$-NH$_2$), 163.6 (HN-*C*O-NH), 167.6 (*C*O-NH).

ESI$^+$-MS: 386.2 (10, [M+Na]$^+$), 365.1 (20, [M+2H]$^{2+}$), 364.1 (100, [M+H]$^+$).

4.3.2. N,N′-Bis-(3,5-di-tert-butylsalicylidene)-3,4-diaminobenzoic acid, aminobiotinyl amide, ***Sal-2***

To a solution of 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (386 mg, 1.65 mmol) in MeOH (10 mL) was added a solution of 3,4-diaminobenzoic acid, aminobiotinyl amide (200 mg, 0.55 mmol) in MeOH (10 mL) and the reaction mixture was refluxed during 48 h. After this time, MeOH was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (initially pure CH_2Cl_2, final mixture 95/5) to afford **Sal-2** as a yellow-orange solid (287 mg, 0.36 mmol, 65% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ_H (ppm) 1.33 (s, 18H, 2 × C(C*H*$_3$)$_3$), 1.45 (s, 18H, 2 × C(C*H*$_3$)$_3$), 1.33–1.67 (m, 8H, C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$-CH$_2$NH), 2.62 (d, *J* = 12.8 Hz, 1H, C*H*$_2$-S), 2.82 (dd, *J* = 12.8 Hz, *J* = 4.7 Hz, 1H, C*H*$_2$-S), 3.07–3.12 (m, 1H, C*H*-S), 3.36–

3.51 (m, 2H, CH_2-NH), 4.19–4.22 (m, 1H, C*H*-N), 4.39–4.42 (m, 1H, C*H*-N), 5.67 (s, 1H; CH-N*H*), 6.68 (s, 1H; CH-N*H*), 7.23–7.27 (m, 3H, *H*C$_{aromatic}$), 7.46 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 7.48 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 7.78 (dd, *J* = 8.2 Hz, *J* = 1.8 Hz, 1H, *H*C$_{aromatic}$), 7.84 (d, *J* = 1.8 Hz, 1H, *H*C$_{aromatic}$), 8.68 (s, 1H, C*H*=N), 8.79 (s, 1H, C*H*=N), 13.41 (s, 1H, O*H*), 13.45 (s, 1H, O*H*).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ_C (ppm) 27.4 (*C*H$_2$), 28.9 (*C*H$_2$), 29.0 (*C*H$_2$), 29.5 (*C*H$_2$), 29.8 (3 × *C*H$_3$), 31.9 (6 × *C*H$_3$), 34.6 (3 × *C*H$_3$), 35.5 (4 × *C*-CH$_3$), 40.3 (*C*H$_2$-NH), 40.9 (*C*H$_2$-S), 56.3 (*C*H-S), 60.5 (*C*H-N), 62.3 (*C*H-N), 118.6 (*C*$_{aromatic}$), 118.7 (*C*$_{aromatic}$), 119.4 (*C*$_{aromatic}$), 120.3 (*C*$_{aromatic}$), 126.3 (*C*$_{aromatic}$), 127.4 (*C*$_{aromatic}$), 127.6 (*C*$_{aromatic}$), 128.9 (*C*$_{aromatic}$), 129.1 (*C*$_{aromatic}$), 133.9 (*C*$_{aromatic}$), 137.5 (*C*$_{aromatic}$), 137.7 (*C*$_{aromatic}$), 141.0 (2 × *C*$_{aromatic}$), 143.0 (*C*$_{aromatic}$), 145.6 (*C*$_{aromatic}$), 159.0 (*C*$_{aromatic}$), 159.1 (*C*$_{aromatic}$), 164.6 (HN-*C*O-NH), 166.0 (2 × *C*H=N), 167.1 (*C*O-NH).

ESI$^+$-MS: 818.6 (10, $[M+Na]^+$), 798.5 (20), 797.4 (50, $[M+2H]^{2+}$), 796.5 (100, $[M+H]^+$).

4.4. Synthesis of N-((5-O-Biotinyl)-2,5-dihydroxysalicylidene), N′-(3,5-di-tert-butylsalicylidene)-ethylenediamine, **Sal-3**

4.4.1. 5-(O-Biotinyl)-2,5-dihydroxybenzaldehyde

2,5-Dihydroxybenzaldehyde (1 g, 7.24 mmol), BNHS (2.06 g, 6.03 mmol) and Et_3N (1.21 mL, 9.05 mmol) were mixed in DMF (30 mL) and the resulting solution was stirred at room temperature for 48 h. After this time, the DMF was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (95/5) to afford the product (437 mg, 1.20 mmol, 20% yield).

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ_H (ppm) 1.57–1.40 (m, 4H, C*H*$_2$C*H*$_2$-CH$_2$CO), 1.63–1.70 (m, 2H, C*H*$_2$-CHS), 2.58 (t, *J* = 7.4 Hz, 2H, C*H*$_2$CO), 2.60 (d, *J* = 12.7 Hz, 1H, C*H*$_2$-S), 2.85 (dd, *J* = 12.7 Hz, *J* = 5.1 Hz, 1H, C*H*$_2$-S), 3.12–3.17 (m, 1H, C*H*-S), 4.14–4.18 (m, 1H, C*H*-N), 4.31–4.34 (m, 1H, C*H*-N), 6.39 (s, 1H, CH-N*H*), 6.48 (s, 1H, CH-N*H*), 7.03 (d, *J* = 12.7 Hz, 1H, *H*C$_{aromatic}$), 7.29 (dd, *J* = 8.9 Hz, *J* = 3.0 Hz, 1H, *H*C$_{aromatic}$), 7.35 (d, *J* = 3.0 Hz, 1H, *H*C$_{aromatic}$), 10.26 (s, 1 H, C*H*O).

^{13}C NMR (100 MHz, DMSO-d_6, 298 K): δ_C (ppm) 25.2 (*C*H$_2$), 28.8 (*C*H$_2$), 28.9 (*C*H$_2$), 34.1 (*C*H$_2$CO), 40.7 (*C*H$_2$-S), 59.2 (*C*H-S), 60.1 (*C*H-N), 61.9 (*C*H-N), 119.1 (*C*$_{aromatic}$H), 121.4 (*C*$_{aromatic}$), 123.3 (*C*$_{aromatic}$), 130.8 (*C*$_{aromatic}$H), 143.6 (*C*$_{aromatic}$), 159.3 (*C*$_{aromatic}$), 163.6 (HN-*C*O-NH), 172.9 (*C*O-O), 191.1 (*C*H=O).

ESI$^+$-MS: 387.1 (100, $[M+Na]^+$), 365.1 (10, $[M+H]^+$).

4.4.2. N-((5-O-Biotinyl)-2,5-dihydroxysalicylidene), N′-(3,5-di-tert-butylsalicylidene)-ethylenediamine, **Sal-3**

To a solution of 5-(*O*-biotinyl)-2,5-dihydroxybenzaldehyde (300 mg, 0.82 mmol) and 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (192 mg, 0.82 mmol) in MeOH (30 mL) was added a solution of Et_3N (53 μL, 0.8 mmol) in MeOH (10 mL) and the resulting reaction mixture was stirred at room temperature for 10 min. The Schiff bases were precipitated by adding water (50 mL) and the solid was collected after centrifugation of the resulting suspension, dissolved in CH_2Cl_2 and dried over $MgSO_4$. After evaporation of the CH_2Cl_2, the three Schiff bases were separated by flash chromatography on silicagel using CH_2Cl_2/MeOH (initially pure CH_2Cl_2, final mixture 97/3) to afford the non-symmetric Schiff base **Sal-3** as a yellow solid (141 mg, 0.23 mmol, 28% yield).

^{1}H NMR (400 MHz, CD_3OD, 298 K): δ_H (ppm) 1.29 (s, 9H, C(C*H*$_3$)$_3$), 1.41 (s, 9H, C(C*H*$_3$)$_3$), 1.42–1.82 (m, 6H, C*H*$_2$C*H*$_2$C*H*$_2$-CH$_2$CO), 2.60 (d, *J* = 12.7 Hz, 1H, C*H*$_2$-S), 2.73 (t, *J* = 7.4 Hz, 2H, C*H*$_2$-CO), 2.95 (dd, *J* = 12.7 Hz, *J* = 4.9 Hz, 1H, C*H*$_2$-S), 3.18–3.27 (m, 1H, C*H*-S), 3.94–3.96 (m, 4H, NH-C*H*$_2$C*H*$_2$-NH), 4.30–4.36 (m, 1H, C*H*-N), 4.48–4.54 (m, 1H, C*H*-N), 6.87 (d, *J* = 7.8 Hz, 1H, *H*C$_{aromatic}$), 7.04 (m, 2H; 2 × *H*C$_{aromatic}$), 7.12 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 7.37 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 8.41 (s, 1H, C*H*=N), 8.42 (s, 1H, C*H*=N).

4.5. Synthesis of N-biotinyl-N′,N″-bis(3,5-di-tert-butylsalicylidene)diethylenetriamine, **Sal-4**

A solution of 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde (150 mg, 0.64 mmol) and ethylenetriamine (34 μL, 0.32 mmol) in toluene (5 mL) was stirred during 4 h at room temperature. The solvent was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (96/4) to afford a yellow solid (171 mg, quantitative yield). As the product hydrolyzed rapidly in solution, the purification was performed very quickly.

To a solution of *N*,*N*′-bis-(3,5-di-*tert*-butylsalicylidene)diethylenetriamine (170 mg, 0.32 mmol) and BNHS (131 mg, 0.38 mmol) in DMF (5 mL) was added Et_3N (134 μL, 1 mmol) and the reaction mixture was heated at 50 °C during 24 h. The solvent was evaporated and the crude product was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH/Et_3N (95/5/2) to afford **Sal-4** as a yellow solid (168 mg, 69% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ_H (ppm) 1.31 (s, 18H, 2 × C(C*H*$_3$)$_3$), 1.45 (s, 18H, 2 × C(C*H*$_3$)$_3$), 1.59–1.76 (m, 6H, C*H*$_2$C*H*$_2$C*H*$_2$-CH$_2$CO), 2.41 (t, *J* = 7.5 Hz, 2H, C*H*$_2$-CO), 2.73 (d, *J* = 12.7 Hz, 1H, C*H*$_2$-S), 2.90 (dd, *J* = 12.7 Hz, *J* = 4.9 Hz, 1H, C*H*$_2$-S), 3.11–3.16 (m, 1H, C*H*-S), 3.68–3.69 (m, 4H, C*H*$_2$-N=CH), 3.76 (t, *J* = 5.9 Hz, 2H, C*H*$_2$-N), 3.84 (t, *J* = 5.9 Hz, 2H, C*H*$_2$-N), 4.26–4.29 (m, 1H, C*H*-N), 4.47–4.50 (m, 1H, C*H*-N), 5.24 (s, 1H, CH-N*H*), 5.68 (s, 1H, CH-N*H*), 7.07 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 7.09 (d, *J* = 2.4 Hz, 1H, *H*C$_{aromatic}$), 7.39–7.40 (m, 2H, *H*C$_{aromatic}$), 8.34 (s, 1H, C*H*=N), 8.37 (s, 1H, C*H*=N), 13.35 (s, 1H, O*H*), 13.69 (s, 1H, O*H*).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ_C (ppm) 25.5 (*C*H$_2$), 28.7 (*C*H$_2$), 29.8 (*C*H$_3$), 29.8 (*C*H$_3$), 31.9 (*C*H$_3$), 33.2 (*C*H$_2$), 34.4 (*C*H$_2$), 34.5 (*C*-CH$_3$), 35.4 (*C*-CH$_3$), 40.9 (*C*H$_2$-S), 48.3 (*C*H$_2$-N), 50.3 (*C*H$_2$-N), 55.7 (*C*H-S), 57.8 (*C*H$_2$-N), 58.8 (*C*H$_2$-N), 60.5 (*C*H-N), 62.1 (*C*H-N), 117.9 (*C*$_{aromatic}$), 118.2 (*C*$_{aromatic}$), 126.4 (*C*$_{aromatic}$H), 126.6 (*C*$_{aromatic}$H), 127.5 (*C*$_{aromatic}$H), 127.9 (*C*$_{aromatic}$H), 137 (*C*$_{aromatic}$), 137.1 (*C*$_{aromatic}$), 140.6 (*C*$_{aromatic}$), 140.8 (*C*$_{aromatic}$), 158.2 (*C*$_{aromatic}$), 158.4 (*C*$_{aromatic}$), 163.7 (HN-*C*O-NH), 168.1 (*C*H=N), 168.4 (*C*H=N), 173.9 (*C*O-NH).

ESI$^+$-MS: 776.4 (20), 763.5 (55, $[M+2H]^{2+}$), 762.6 (100, $[M+H]^+$).

4.6. Preparation of the manganese-salen complexes Mn-**Sal-1**–Mn-**Sal-4**

4.6.1. (meso)-N-Biotinyl-3,4-(N′,N″-bis(3,5-di-tert-butylsalicylidene))-pyrrolidine, manganese^{III} chloride complex, Mn-**Sal-1**

This synthesis was adapted from a published procedure [41]. To a solution of Mn(OAc)$_2$ 4H$_2$O (44 mg, 0.18 mmol) in EtOH (1.5 mL) was added a solution of **Sal-4** in toluene (1.8 mL) and the resulting mixture was refluxed for 1 h. Saturated aqueous NaCl (0.2 mL) was then added and air was bubbled through the solution during 30 min. The reaction mixture was cooled to room temperature, toluene (4 mL) and CH_2Cl_2 (10 mL) were added and the organic phase was separated and washed two times with water. The aqueous phase was extracted with CH_2Cl_2 until it was colourless and the collected organic layers were dried over Na_2SO_4 and evaporated under vacuum. The Mn complex was purified by flash chromatography on silicagel using CH_2Cl_2/MeOH (75/15) to afford a brown solid (28.5 mg, 56% yield). ESI$^+$-MS: 812.5 (100, $[M-Cl]^+$), 813.5 (40, $[M-Cl+H]^+$), 814.3 (10, $[M-Cl+2H]^+$).

4.6.2. Mn-Sal-2–Mn-Sal-4

The three Mn-**Sal-X** complexes (X = 2, 3 or 4) were prepared by mixing solutions of known concentrations of Mn(OAc)$_2$ 4H$_2$O (0.08 M) and **Sal-X** (0.04 M) in DMF (1/1 volumes of the two solu-

tions) and heating the resulting mixtures in closed tubes at 65 °C. Thus, the corresponding Mn-**Sal-X** complexes were available in 0.02 M solutions in DMF and were used without further isolation and purification.

4.7. Typical procedure for the sulfoxidation of thioanisole

4.7.1. Using Mn-***Sal-1*** *as catalyst (Table 1)*

A solution of streptavidin in water (400 μL, 60 μM tetrameric concentration) was mixed in a test tube with the biotinylated metal complex, Mn-**Sal-1** (4 μL of a 0.02 M stock solution in DMF, 200 μM final concentration). After 5 min incubation time, thioanisole was added (4 μL of a 1 M DMF stock solution). The reaction was initiated by adding 1 equivalent of H_2O_2 and the test tube was sealed. After 4–5 h stirring at room temperature, the reaction mixture was extracted four times with Et_2O. The organic phase was dried over Na_2SO_4 and subjected to HPLC analysis using a chiral OD-H column (Daicel Chemical Industries, Tokyo) with hexane:isopropanol 90:10 at 0.8 mL/min; t_R = 13.1 min, t_S = 17.1 min (UV detection at 215 nm). The absolute configuration of the sulfoxides was assigned according to published results [32].

4.7.2. Using Mn-Sal-2–Mn-Sal-4 as catalysts (Table 2)

A solution of streptavidin in water (400 μL, 33 μM tetrameric concentration) was mixed in a test tube with the biotinylated metal complex, Mn-**Sal-X** (2 μL of a 0.02 M stock solution in DMF, 100 μM final concentration). After 5 min incubation time, thioanisole was added (2 μL of a 1 M DMF stock solution). The reaction was initiated by adding 1 equivalent of H_2O_2 and the test tube was sealed. After 4–5 h stirring at room temperature, the reaction mixture was extracted four times with Et_2O. The organic phase was dried over Na_2SO_4 and subjected to HPLC analysis using a chiral OD-H column (Daicel Chemical Industries, Tokyo) with hexane:isopropanol 90:10 at 0.8 mL/min; t_R = 13.1 min, t_S = 17.1 min (UV detection at 215 nm). The absolute configuration of the sulfoxides was assigned according to published results [32].

Acknowledgements

This work was supported by the Canton of Neuchâtel, and the Swiss National Science Foundation (Grants FN 200021-105192 and 200020-113348), we thank Prof. C.R. Cantor for providing us with the streptavidin gene.

References

[1] A.J. Thomson, H.B. Gray, Curr. Opin. Chem. Biol. 2 (1998) 155.
[2] R.R. Davies, M.D. Distefano, J. Am. Chem. Soc. 48 (1997) 11643.
[3] M.E. Wilson, G.M. Whitesides, J. Am. Chem. Soc. (1978) 306.
[4] C.-C. Lin, C.-W. Lin, A.S.C. Chan, Tetrahedron: Asymmetr. (1999) 1887.
[5] J. Collot, J. Gradinaru, N. Humbert, M. Skander, A. Zocchi, T.R. Ward, J. Am. Chem. Soc. 30 (2003) 9030.
[6] M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni, T.R. Ward, J. Am. Chem. Soc. 44 (2004) 14411.
[7] G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U.E. Rusbandi, T.R. Ward, Angew. Chem., Int. Ed. 47 (2005) 7764.
[8] U.E. Rusbandi, C. Lo, M. Skander, A. Ivanova, M. Creus, N. Humbert, T.R. Ward, Adv. Synth. Catal. 11–12 (2007) 1923.
[9] M.T. Reetz, J.J.-P. Peyeralans, A. Maichele, Y. Fu, M. Maywald, Chem. Commun. (2006) 4318.
[10] H. Yamaguchi, T. Hirano, H. Kiminami, D. Taura, A. Harada, Org. Biomol. Chem. (2006) 3571.
[11] L. Panella, J. Broos, J. Jin, M.W. Fraaije, D.B. Janssen, M. Jeronimus-Stratingh, B.L. Feringa, A.J. Minnaard, J.G. deVries, Chem. Commun. (2005) 5656.
[12] C. Letondor, N. Humbert, T.R. Ward, Proc. Natl. Acad. Sci. USA 13 (2005) 4683.
[13] C. Letondor, A. Pordea, N. Humbert, A. Ivanova, S. Mazurek, M. Novic, T.R. Ward, J. Am. Chem. Soc. 25 (2006) 8320.
[14] M. Creus, A. Pordea, T. Rossel, A. Sardo, C. Letondor, A. Ivanova, I. Le Trong, R.E. Stenkamp, T.R. Ward, Angew. Chem., Int. Ed. (2008) 1400.
[15] T. Kokubo, T. Sugimoto, T. Uchida, S. Tanimoto, M. Okano, J. Chem. Soc., Chem. Commun. 14 (1983) 769.
[16] K. Okrasa, R.J. Kazlauskas, Chem. Eur. J. 6 (2006) 1587.
[17] A. Fernández-Gacio, A. Codina, J. Fastrez, O. Riant, P. Soumillion, ChemBioChem 7 (2006) 1013.
[18] F. van de Velde, L. Könemann, F. van Rantwijk, R.A. Sheldon, Chem. Commun. 17 (1998) 1891.
[19] M. Ohashi, T. Koshiyama, T. Ueno, M. Yanase, H. Fujii, Y. Watanabe, Angew. Chem., Int. Ed. 9 (2003) 1005.
[20] T. Ueno, T. Koshiyama, M. Ohashi, K. Kondo, M. Kono, A. Suzuki, T. Yamane, Y. Watanabe, J. Am. Chem. Soc. (2005) 6556.
[21] J.R. Carey, S.K. Ma, T.D. Pfister, D.K. Garner, H.K. Kim, J.A. Abramite, Z. Wang, Z. Guo, Y. Lu, J. Am. Chem. Soc. (2004) 10812.
[22] A. Mahammed, Z. Gross, J. Am. Chem. Soc. (2005) 2883.
[23] A. Pordea, M. Creus, J. Panek, C. Duboc, D. Mathis, M. Novic, T.R. Ward, J. Am. Chem. Soc. 25 (2008) 8085.
[24] M.T. Reetz, N. Jiao, Angew. Chem., Int. Ed. (2006) 2416.
[25] J. Pierron, C. Malan, M. Creus, J. Gradinaru, I. Hafner, A. Ivanova, A. Sardo, T.R. Ward, Angew. Chem., Int. Ed. 4 (2008) 701.
[26] C.M. Thomas, C. Letondor, N. Humbert, T.R. Ward, J. Organomet. Chem. 20 (2005) 4488.
[27] J.F. Larrow, E.N. Jacobsen, Top. Organomet. Chem. Organomet. Process Chem. (2004) 123.
[28] T. Katsuki, Adv. Synth. Catal. 2 (2002) 131.
[29] E.M. McGarrigle, D.G. Gilheany, Chem. Rev. 5 (2005) 1563.
[30] K. Matsumoto, Y. Sawada, B. Saito, K. Sakai, T. Katsuki, Angew. Chem., Int. Ed. 31 (2005) 4935.
[31] T. Yamaguchi, K. Matsumoto, B. Saito, T. Katsuki, Angew. Chem., Int. Ed. 25 (2007) 4729.
[32] H. Egami, T. Katsuki, J. Am. Chem. Soc. (2007) 8940.
[33] Y. Sawada, K. Matsumoto, S. Kondo, H. Watanabe, T. Ozawa, K. Suzuki, B. Saito, T. Katsuki, Angew. Chem., Int. Ed. 21 (2006) 3478.
[34] K. Noda, N. Hosoya, K. Yanai, R. Irie, T. Katsuki, Tetrahedron Lett. 12 (1994) 1887.
[35] M. Palucki, P. Hanson, E.N. Jacobsen, Tetrahedron Lett. 47 (1992) 7111.
[36] C. Kokubo, T. Katsuki, Tetrahedron 44 (1996) 13895.
[37] B.D. Brandes, E.N. Jacobsen, J. Org. Chem. 16 (1994) 4378.
[38] T. Hamada, T. Fukuda, H. Imanishi, T. Katsuki, Tetrahedron 2 (1996) 515.
[39] T. Hashihayata, Y. Ito, T. Katsuki, Tetrahedron 28 (1997) 9541.
[40] E.N. Jacobsen, W. Zhang, A.R. Muci, J.R. Ecker, L. Deng, J. Am. Chem. Soc. (1991) 7063.
[41] J.F. Larrow, E.N. Jacobsen, Y. Gao, Y. Hong, X. Nie, C.M. Zepp, J. Org. Chem. 7 (1994) 1939.
[42] T. Schwenkreis, A. Berkessel, Tetrahedron Lett. 30 (1993) 4785.
[43] P. Pietikäinen, Tetrahedron Lett. 6 (1994) 941.
[44] D.S. Wilbur, M.-K. Chyan, P.M. Pathare, D.K. Hamlin, M.B. Frownfelter, B.B. Kegley, Bioconjugate Chem. 4 (2000) 569.
[45] M. Wilchek, E.A. Bayer (Eds.), Methods in Enzymology: Avidin–Biotin Technology, Academic Press, San Diego, 1990.
[46] M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni, T.R. Ward, J. Am. Chem. Soc. (2004) 14411.
[47] M. Couturier, B.M. Andresen, J.B. Jorgensen, J.L. Tucker, F.R. Busch, S.J. Brenek, P. Dube, D.J. Ende, J.T. Negri, Org. Process Res. Dev. 1 (2002) 42.
[48] A.M.D.A. Rocha Gonsalves, M.E.S. Serra, D. Murtinho, V.F. Silva, A. Matos Beja, J.A. Paixao, M. Ramos Silva, L. Alte Da Veiga, J. Mol. Catal. A: Chem. 1–2 (2003) 1.
[49] G.L. Baker, S.J. Fritschel, J.R. Stille, J.K. Stille, J. Org. Chem. 14 (1981) 2954.
[50] B.M. Choudary, N.S. Chowdari, M.L. Kantam, P.L. Santhi, Catal. Lett. 3–4 (2001) 213.
[51] R.G. Konsler, J. Karl, E.N. Jacobsen, J. Am. Chem. Soc. 41 (1998) 10780.