STRUCTURAL INVESTIGATIONS OF

amavadin-BASED VANADIUM COMPLEXES

TON HUBREGTSE

STRUCTURAL INVESTIGATIONS on AMAVADIN-BASED VANADIUM COMPLEXES

T. HUBREGTSE 2007

UITNODIGING

UITNODIGING VOOR HET BIJWONEN VAN DE OPENBARE VERDEDIGING VAN HET PROEFSCHRIFT EN DE STELLINGEN OP

DINSDAG 17 APRIL 2007 OM 12.30 UUR

IN DE SENAATSZAAL VAN DE TECHNISCHE UNIVERSITEIT DELFT
MEKELWEG 5 | DELFT

Voorafgaand aan de verdediging is er om 12.00 uur een toelichting voor niet-chemici.

Na afloop van de plechtigheid bent u van harte welkom op de receptie in hetzelfde gebouw.

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Propositions

belonging to the thesis 'Structural investigations of amavadin-based vanadium complexes'

by Ton Hubregtse

1. Strongly acidic ion-exchange resins should not be used to purify molecules that rely on non-covalent bonds.

   E. M. Armstrong; D. Collison; N. Ertok; C. D. Garner, *Talanta*, **2000**, *53*, 75–87.
   H. Kneifel; E. Bayer, *J. Am. Chem. Soc.*, **1986**, *108*, 3075–3077.

2. The rule that states that the exception proves the rule is proven because there is no exception to this rule that can prove it.

3. The NMR data of Smith *et al.* can much better be explained when it is considered, given the three possible ligand stereoisomers and the two possible vanadium configurations, that six enantiomeric pairs of the [V(*R*,*S*-hidba)$_2$]-anion can be formed instead of three.

   P. D. Smith *et al.*, *J. Chem. Soc., Dalton Trans.*, **1997**, 4509–4516.

4. Fitness-equipment that does not make you tired and magical potions for improving your physical appearance are bestsellers because people expect to have bought the solution for their lack of discipline.

5. The determination of the number of water molecules in a sample by elemental analysis is not a reliable method.

   Chapter 5 of this thesis.

6. The term 'conformational isomers', used by Deutsch *et al.* for the indication of the reaction products of glycerol and benzaldehyde is incorrect.

   J. Deutsch; A. Martin; H. Lieske, *Journal of Catalysis*, **2007**, *245*, 428–435.

7. Without dreaming of the future one will never see opportunities.

8. The authors of the reported one-pot synthesis of acetic acid from methane and trifluoracetic acid (as CO-donor and solvent) should first consider the atom efficiency and the fluorinated by-products before comparing their synthesis with the industrial process.

   P. M. Reis *et al.*, *Angew. Chem. Int. Ed.*, **2003**, *42*, 821–823.

9. It can not be proven that the biological homochirality on Earth is of extraterrestrial origin.

   J. Bailey *et al.*, *Science*, **1998**, *281*, 672–674.

10. Speed skating is the only sport where one can become a world champion with two hands at the back.

These propositions are considered opposable and defendable and as such have been approved by the supervisor, Prof. dr. R.A. Sheldon.

Stellingen

behorende bij het proefschrift 'Structural investigations of amavadin-based vanadium complexes'

van Ton Hubregtse

1. Sterk-zure ionenwisselaars zouden niet gebruikt moeten worden om moleculen te zuiveren die non-covalente bindingen hebben.

   E. M. Armstrong; D. Collison; N. Ertok; C. D. Garner, *Talanta*, **2000**, *53*, 75–87.
   H. Kneifel; E. Bayer, *J. Am. Chem. Soc.*, **1986**, *108*, 3075–3077.

2. De regel die zegt dat de uitzondering de regel bevestigt, wordt bevestigd doordat hij geen uitzondering kent die hem kan bevestigen.

3. De NMR-resultaten van Smith *et al*. zijn een stuk beter te verklaren als in ogenschouw wordt genomen dat er, gegeven de drie mogelijke ligandstereoisomeren en de twee mogelijke vanadiumconfiguraties, zes enantiomere paren van het $[V(R,S\text{-hidba})_2]$ anion kunnen ontstaan en geen drie.

   P. D. Smith *et al.*, *J. Chem. Soc., Dalton Trans.*, **1997**, 4509–4516.

4. Fitnessapparaten waarop je niet moe wordt en wondermiddeltjes voor een mooi uiterlijk verkopen zo goed omdat men verwacht de oplossing te hebben gekocht voor gebrek aan discipline.

5. Het bepalen van het aantal watermoleculen in een monster met behulp van elementanalyse is geen betrouwbare methode.

   Hoofdstuk 5 van dit proefschrift.

6. De term 'conformational isomers', gebruikt door Deutsch *et al*. ter aanduiding van de reactieproducten van glycerol en benzaldehyde is onjuist.

   J. Deutsch; A. Martin; H. Lieske, *Journal of Catalysis*, **2007**, *245*, 428–435.

7. Zonder dromen over de toekomst zul je nooit kansen zien.

8. De auteurs van de beschreven één-pots-synthese van azijnzuur uit methaan en trifluorazijnzuur (als CO-donor en oplosmiddel) zouden eerst de atoom-efficiëntie en de gefluoreerde bijproducten in beschouwing moeten nemen alvorens hun synthese met het industriële proces te vergelijken.

   P. M. Reis *et al.*, *Angew. Chem. Int. Ed.*, **2003**, *42*, 821–823.

9. Het is niet te bewijzen dat de biologische homochiraliteit op Aarde van buitenaardse oorsprong is.

   J. Bailey *et al.*, *Science*, **1998**, *281*, 672–674.

10. Hardrijden op de schaats is de enige sport waarbij je met twee handen op de rug wereldkampioen kunt worden.

Deze stellingen worden opponeerbaar en verdedigbaar geacht en zijn als zodanig goedgekeurd door de promotor, Prof. dr. R.A. Sheldon.

Structural investigations of amavadin-based vanadium complexes

Cover design by Taeke Castelein (www.taekecastelein.nl)

Structural investigations of amavadin-based vanadium complexes

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof. dr. ir. J.T. Fokkema,
voorzitter van het College voor Promoties,
in het openbaar te verdedigen op dinsdag 17 april 2007 om 12.30 uur

door

Teunis HUBREGTSE
doctorandus in de scheikunde
geboren te Winschoten

Dit proefschrift is goedgekeurd door de promotor:
Prof. dr. R. A. Sheldon

Samenstelling promotiecomissie:

Rector Magnificus	voorzitter
Prof. dr. R. A. Sheldon	Technische Universiteit Delft, promotor
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Reservelid:
Prof. dr. ir. H. van Bekkum

Dr. U. Hanefeld en Prof. dr. I.W.C.E. Arends hebben als begeleiders in belangrijke mate aan de totstandkoming van dit proefschrift bijgedragen.

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Aan mijn vrouw
Yvonne

Aan mijn opa
Ton Hubregtse (“grote Ton”)

Die eerste inslag zal je maatstaaf zijn. Alle inslagen die volgen zul je eraan afmeten.

Dirk Ayelt Kooiman

Contents

1 INTRODUCTION

1.1. History, occurrence and properties of vanadium

Vanadium was originally discovered by the mineralogist Andrés Manuel del Río (1764–1849) in a mine in northern Mexico in 1801.[1,2] He believed that he had discovered the previously unknown element 23 in a sample of 'brown lead', now known as vanadinite ($Pb_5(VO_4)_3Cl$). Because the colours of this mineral were reminiscent of those shown by chromium, he called the new element *panchromium*. He later renamed it *erythronium* ('red') as a reference to the red colour observed when the mineral was treated with acids. Unfortunately, he withdrew his claim when it was suggested by the French chemist Hippolyte-Victor Collet-Descotils (1773–1815) that the mineral was only impure lead chromate. The element was 'rediscovered' in 1831 by the Swedish chemist Nils Gabriel Sefström (1787–1845) in remnants of iron ore at the Taberg mine in Sweden. He called the 'new element' *vanadium*, in honour of Vanadis, the Scandinavian goddess of love, beauty and youth. In the same year, Friedrich Wöhler (1800–1882) came into possession of del Río's 'brown lead' and he established that the 'new element' of Sefström was identical to the one del Río had reported three decades earlier. Though, the name vanadium still stands rather than del Río's suggestion of erythronium. Metallic vanadium was not isolated until 1867 when Henry Enfield Roscoe (1833–1915) reduced vanadium chloride with hydrogen gas to give vanadium metal. He was also responsible for much of the early work on the element.

Vanadium has been estimated to comprise about 136 ppm of the earth's crustal rocks, in which it is the 19th most abundant element and the fifth most abundant transition metal after iron, titanium, manganese and zirconium. Although vanadium is a common and widespread element, it is sparsely distributed; it is not mined as such but is generally obtained as a by-product of other ores and it is recovered from some crude oils, in particular those from Venezuela and Canada. The concentration of vanadium in the world's oceans is about 20–35 nM, making it the second most abundant transition metal in the aquasphere. It is only surpassed by molybdenum (100 nM), while it is clearly more abundant than iron (0.02–1 nM).[3] Vanadium is commonly used as an additive to steel and in different catalysts for various industrial oxidation purposes. This kind of application of vanadium as well as the burning of fossil fuels have resulted in a significant enrichment of the environment with vanadium.

Vanadium[4] is a shiny, silvery metal, of which the pure form is soft and ductile. It resists corrosion due to a protective film of oxide on the surface and has a melting point of 1915 °C. Being a group V element in the first transition series with the atomic number 23, vanadium has the ground state electronic configuration of $[Ar]4s^23d^3$. Natural vanadium is a mixture of two isotopes, ^{51}V (99.75%) and ^{50}V (0.25%), the latter being radioactive with a half-life of $>3.19 \times 10^{17}$ years. Almost all vanadium NMR is performed on the abundant ^{51}V nucleus,[5] which has

a nuclear spin of $I = 7/2$. The nuclear electric quadrupole moment of ^{51}V is unusually low, which makes its spectra easier to observe and resolve. The chemical shifts of the ^{51}V nucleus span a range of over 4000 ppm and are very sensitive to the nature of the coordination sphere of the metal as well as to its oxidation state. These features make NMR a useful tool for the characterisation of vanadium complexes.

Vanadium is known to exist in eight oxidation states ranging from –3 to +5, with the exception of –2 (although there is no reason to believe that such compounds cannot exist).[6] In the biosphere, the oxidation states +2,+3, +4 and +5 are found, but the +4 and the +5 are the most common ones. The coordination chemistry of vanadium(IV) and (V) compounds is dominated by oxo-complexes,[7] in which O^{2-} is the formal ligand. The majority of vanadium(IV) compounds contain the uniquely stable vanadyl ion (VO^{2+}), which retains its identity throughout a wide variety of reactions. When coordinated, vanadium(V) is usually present as the oxidized vanadyl (VO^{3+}) or as the pervanadyl (VO_2^{+}) moiety. In a few cases, vanadium(IV) and (V) *non*-oxo complexes have been reported. These compounds lack the oxo ligand on the vanadium, which is then referred to as 'bare vanadium' or 'naked vanadium'.[8] Due to their d^1 electronic configuration, vanadium(IV) species can easily be identified by EPR spectroscopy, whereas the d^0 vanadium(V) nuclei are EPR silent.

1.2. Vanadium in biological systems

The importance of vanadium for living organisms was first recognized in 1911 by Henze,[9] who found high contents of vanadium in the blood of tunicates. Whereas during the previous century much progress was made on the understanding of the biological role of other metals,[10] the importance of vanadium for living organisms[11,12,13] has become generally established only in the last twenty-five years. At present, the areas of vanadium involvement in biological systems[14,15,16] can be summarized under the following five headings:[17,18]

(1) Vanadates as phosphate analogues

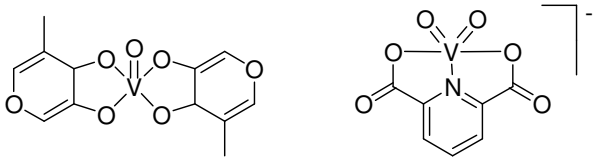
Vanadate (VO_4^{3-}) has long been recognized as a structural and electronic analogue of phosphate (PO_4^{3-}). Given the central role of phosphate in biology, a wide range of physiological effects of vanadates and vanadate derivatives can be envisioned. Two cases can be distinguished:

1) The inhibition of many phosphorylase enzymes like phosphatases, ribonucleases and ATPases by vanadate.
2) The recognition by proteins of vanadate, vanadate esters and vanadate anhydrides as alternate substrates.

1) Inhibition of enzymes

The potent inhibition of many phosphorylase enzymes relies on the analogy of five-coordinate vanadium compounds with the transition state of phosphate ester hydrolysis.[19] Phosphoryl transfer reactions of the four-coordinate phosphate are believed to proceed via a high energy five-coordinate phosphate transition state. Phosphorylase enzymes catalyze their reactions by stabilizing this high energy state. Vanadate can adopt five-coordinate structures readily, thus it can be expected that vanadate binds tightly to some phosphorylase enzymes.[20] In this way, inhibition of the enzyme can be accomplished, even at low vanadate concentrations. There is strong evidence that the observed biological activities of vanadium already occur at 10^{-5}–10^{-7} M vanadium concentrations, which is close to the 10^{-6}–10^{-9} M concentrations found in tissues of mammals and plants.[21] The regulation of phosphate-metabolizing enzymes by vanadium compounds seems to be a general function, which makes vanadium a trace metal possibly essential for all organisms.

The physiological action of vanadium compounds has also fueled investigations into medical applications.[22,23] The therapeutic effects of vanadium include anti-cancer and anti-inflammatory activities,[24] but the main focus has been on its activity in the treatment of diabetes.[25,26] The effects exerted by vanadium compounds are commonly referred to as 'insulin-enhancing' or 'insulin-mimetic'. Initially, vanadium salts were believed to be a substitute for insulin, but their action appeared to be more subtle. The insulin signaling system is extremely complex, and the insulin-enhancing effect of vanadate is generally believed to involve competitive inhibition of regulatory protein phosphatases.[27] Vanadium probably has the oxidation state of +4 at the *in vivo* target, but studies in this direction are difficult because in cellular studies scientists generally have lost control over the environment of the compound. Despite the well established potential of vanadium compounds in the treatment of diabetes,[28] little progress has been made in the transfer into a viable drug. Currently, only one compound has been introduced into clinical tests with humans, which is bis(maltolato)oxovanadium(IV) **1** (Figure 1.1). In addition, dipicolinato-oxovanadium(V) **2** has been successfully used to treat diabetic cats. Whereas most of the active compounds contain vanadium in oxidation state +4 upon administration, **2** is the first well-characterized vanadium(V) compound with insulin-like properties.



1 **2**

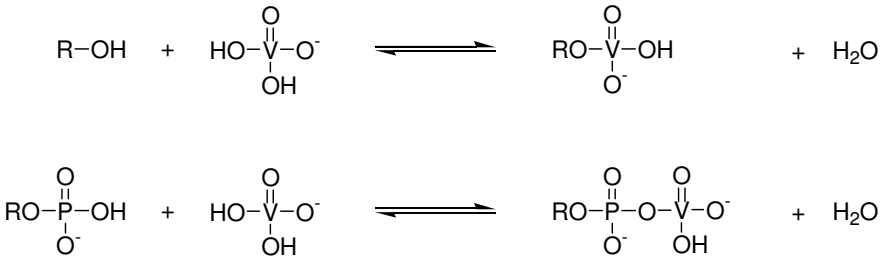
Figure 1.1. *Two vanadium compounds for which insulin enhancing behaviour has been established.*

A new family of picolinatovanadium compounds was introduced in 2003 by the group of Rehder. The ligands are monoesters and monocarboxylates of 2,5-dipicolinic acid; the modification of the ester moiety allows a fine tuning of the lipophilicity/hydrophilicity of the complexes and also enables the introduction of organic molecules for which membrane receptors exist.[29,30]

The reluctance of pharmaceutical companies to bring vanadium-based medicines to the market can mainly be attributed to the toxicity of the compounds. It appeared that especially the simple vanadium salts have a very narrow therapeutic window and therefore research towards complexes of vanadium is important. The fear of a drug containing a 'heavy metal' probably also plays a role in its acceptance by the market. In this respect, it is interesting to note that vanadium in the form of $VOSO_4$ is used by bodybuilders as a diet supplement to enhance performance and muscle growth. However, in research on the effectiveness of this compound no benefits were found upon administration of dosages of more than 1000 times the nutritional dosage.[31]

2) Recognition by proteins

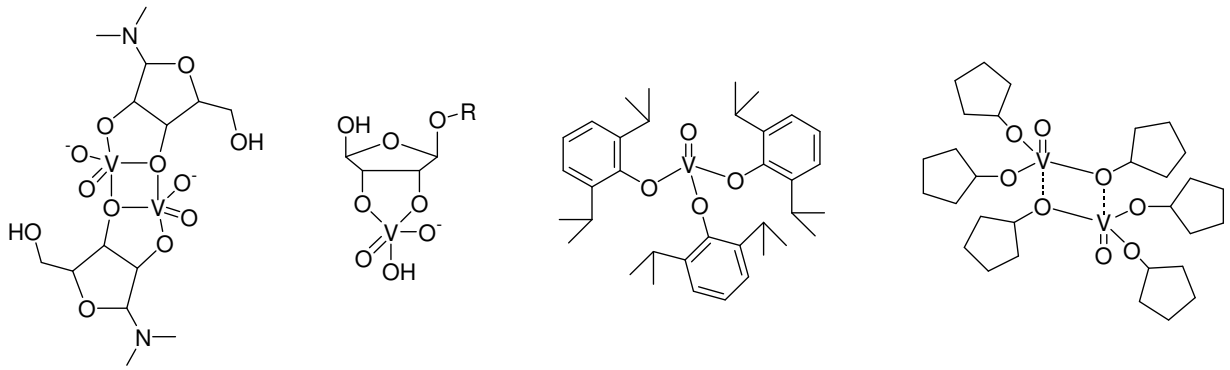
Two of the most important cellular metabolites are phosphate esters and phosphate anhydrides, for which the vanadate mimicks are vanadate esters and vanadate anhydrides, respectively.[18] Application of the vanadate-phosphate analogy has been successful with enzymes that catalyze the formation of phosphate esters and phosphate anhydrides, which opened the possibility of forming vanadium analogues as shown in Scheme 1.1.



Scheme 1.1. *Application of the vanadate-phosphate analogy opened the possibility of forming vanadium analogues of phosphate esters and phosphate anhydrides.*

The interaction between vanadate, enzymes and enzyme substrates has stimulated investigations to elucidate the structures, kinetics and mechanisms that play a role in these reactions. A range of model systems was used to simulate the binding of vanadate to enzyme functionalities and enzyme substrates. Examples of these models are the proposed vanadate containing structures **3**[32,33] and **4**[34] (Figure 1.2). For further examination of the vanadate-phosphate analogy, many vanadate derivatives were prepared to evaluate the effects of ligands on the vanadium geometry. Two examples are the vanadate esters **5** and **6**[18] (Figure 1.2).

These respective four- and five-coordinate structures also serve the investigations towards transition state mimics of phosphorylase reactions.

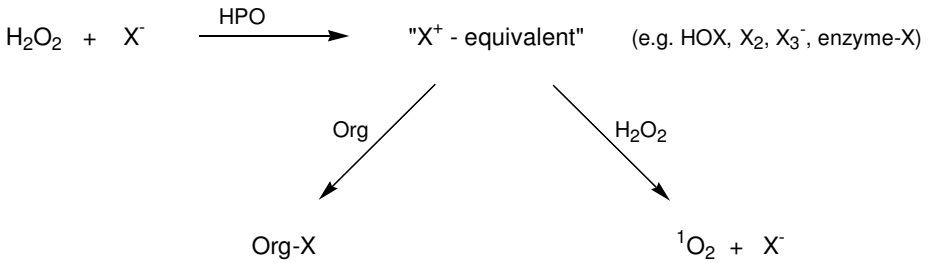


3 **4** **5** **6**

Figure 1.2. *Model compounds that were prepared with the aim of simulating the binding of vanadate (**3**, **4**) and evaluating the effects of ligands on the vanadium geometry (**5**, **6**).*

(2) Vanadium dependent haloperoxidases

Vanadium haloperoxidases (V-HPO's) are vanadium(V)-containing enzymes which have been isolated from several brown algae, some red algae, one green alga, one fungus and one terrestrial lichen.[35,36] They catalyze the two-electron oxidation of a halide by hydrogen peroxide to the corresponding hypohalous acids[37] (Scheme 1.2).



Scheme 1.2. *The HPO-catalyzed oxidation of a halide by hydrogen peroxide.*

The enzymes are named after the most electronegative halide ion they are able to oxidize; vanadium chloroperoxidase (V-CPO) oxidises Cl^-, Br^- and I^-, while vanadium bromoperoxidase (V-BrPO) oxidizes Br^- and I^-. The oxidized halide equivalent (*i.e.* HOX, X_2 or X_3^-) can then halogenate an organic substrate. In this way, V-HPO's (and HPO's in general) are thought to play a role in the biosynthesis of numerous halogenated marine natural products.[38] The significance of these compounds is not entirely understood; it is assumed that they are part of the host defense system of the marine species. It is recognized that the volatile chlorinated and brominated hydrocarbons are produced in enormous quantities, which can certainly have global effects, most notably on the ozone layer. After the first isolation of V-

BrPO in 1984[39] from the marine brown alga *Ascophyllum Nodosum*, several V-HPO's have been detected and isolated[35] and the enzyme mechanism and structure were examined.[18,40,41] It is suggested that the oxidation state of the vanadium remains +5 and does not change during catalysis. Furthermore, model complexes of V-HPO's were prepared to explore the medicinal and catalytic potential of this catalytic system.[42] Another interesting feature of V-HPO's is their phosphatase activity after removal of the vanadate.[43] The discovered analogy with respect to structural as well as catalytic features between these classes of enzymes reflects their comparable affinities for vanadate. On the other hand, peroxidase activity has been observed for vanadate-inhibited phosphatases; for example, sulfoxidation activity has also been observed with phytase from *Aspergillus ficuum*, when vanadate and hydrogen peroxide were added.[44]

The observed stability of V-HPO's in the presence of high concentrations of strong oxidants or organic solvents and their stability at elevated temperatures makes them attractive as industrial biocatalysts. Another potential commercial application of V-HPO's is their use in environmentally friendly antifouling paints. In an ultimate set-up, a haloperoxidase containing coating on the outer walls of marine vehicles prevents these walls from biofouling, making use of the natural presence of hydrogen peroxide in seawater.[45] An important problem still to overcome is to match the pH optimum of the enzyme with the pH in the oceans.[46]

(3) Accumulation of vanadium in *Amanita muscaria* toadstools

In 1931, it was discovered by Ter Meulen[47] that the toadstool *Amanita muscaria* (fly agaric) contains high levels of vanadium. Since then, vanadium accumulation has been found in a few other species of the genus *Amanita*, some of which were found to contain levels of up to 400 times those typically found in plants.[48,49,50] In 1972, a blue vanadium-containing compound was isolated from *Amanita muscaria* by Kneifel and Bayer,[51] who named it 'amavadin'. Later investigations showed that this compound has the relatively low molecular weight of 401 and that it contains one non-oxo vanadium(IV) centre that is surrounded by two tridentate ligands. HPLC and ESR measurements on samples of *Amanita regalis* and *Amanita velapites* showed that the vanadium in these species is also present as amavadin.[52] In section 1.4, a more detailed description of amavadin is given.

(4) Accumulation of vanadium in the animal kingdom: ascidians and the fan worm

There are only few organisms in the animal kingdom that are known to accumulate vanadium. The most prominent example are the tunicates, a family of invertebrate marine organisms that are also referred to as ascidians or sea squirts. They were already reported in 1911[9] to accumulate high levels of vanadium in their blood, and later investigations have shown that these levels can be up to 1×10^7 times the concentration in sea water.[53] The vanadium is stored in so-called 'vanabins' (=vanadium binding protein) in which it has been shown to be

present in the +3 as well as in the +4 oxidation state. Some of these proteins have been isolated and characterized, and also the mechanism of accumulation and reduction to V(III) and V(IV) have been investigated (vanadium is usually in the +5 oxidation state when taken up from the marine environment). There are a few hypotheses on the natural function of vanadium in tunicates, but none of the proposals seems to be supported by sufficient evidence. More recently, it has been reported that the fan worm *Pseudopotamilla occelata* also accumulates vanadium.[54] The place and form of vanadium storage in these species shows similarities to those in tunicates.[18]

(5) Vanadium dependent nitrogenases

The reduction of dinitrogen to ammonia by nitrogenase enzymes (Scheme 1.3) is called nitrogen fixation, as it makes the dinitrogen from the atmosphere available for the biosynthesis of nucleic acids, amino acids and other nitrogen containing compounds. Therefore, it ranks with photosynthesis as one of the fundamental processes enabling life on earth.[10]

$$N_2 + 6e^- + 6H^+ \xrightarrow{\text{nitrogenase}} 2NH_3$$

Scheme 1.3. *The reduction of dinitrogen to ammonia.*

For many years, nitrogenase enzymes were believed to rely completely on molybdenum, but in the last decades it has become apparent that biological nitrogen fixation also occurs via nitrogenases that are dependent on vanadium.[55,13] The vanadium nitrogenases are distinct enzymes and do not result simply from the substitution of molybdenum by vanadium in the more commonly found molybdenum nitrogenases. In addition to the vanadium haloperoxidases and the vanabins, the vanadium nitrogenases represent the third class of proteins that bind vanadium naturally. The enzyme is constituted of an Fe protein and a V-Fe protein. Its structure is still not known, but EXAFS studies have shown that the vanadium is in an environment similar to that observed for the vanadium in the cage-like $[VFe_3S_4]^{2+}$ unit, where all eight atoms occupy one corner of the cage.

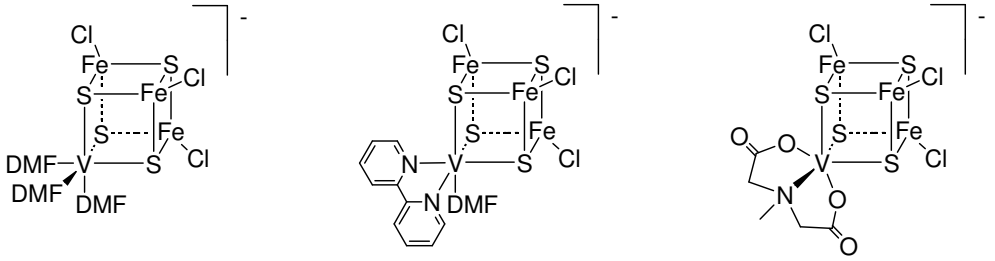


Figure 1.3. *Model compounds of the V-Fe unit in vanadium dependent nitrogenase.*

Another characteristic feature is the absence of an oxo-ligand on the vanadium. To date, the $[VFe_3S_4]^{2+}$ units and the compound 'amavadin' are the only naturally occurring compounds that have been demonstrated to contain a non-oxo vanadium atom. The stability of the $[VFe_3S_4]^{2+}$ unit has been illustrated by model compounds,[55,56] some of which are shown in Figure 1.3. The vanadium oxidation state in the metal clusters of the nitrogenase has not exactly been determined, but it is thought to vary from +2 to +4.

Considerations with respect to vanadium in biological systems

The explosive growth of the chemistry and biochemistry of vanadium compounds since the early eighties was mainly fueled by the discoveries described under headings (1) and (2). At present, the vanadium accumulation in plants and animals as well as the vanadium nitrogenases as described under headings (3) to (5) are not well understood, but efforts to elucidate the properties of these natural systems are ongoing. Moreover, in addition to these five areas of vanadium involvement in biological systems a sixth one is arising: vanadium containing nitrate reductases (Scheme 1.4).[57,58] Almost all nitrate reductases that have been described contain a molybdenum cofactor. However, recently two new nitrate reducing enzymes have been discovered that contain vanadium and lack a molybdenum cofactor. The nitrate reductase isolated from *Pseudomonas isachenkovii* bacteria showed a correlation between increasing nitrate reductase activity and vanadium content, whereas in the nitrate reductase from *Thioalkalivibrio nitratireducens* both vanadium and heme groups are present. The exact coordination of vanadium in these enzymes is not yet elucidated, nor is the oxidation state of the vanadium known.

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate reductase}} NO_2^- + H_2O$$

Scheme 1.4. *The reduction of nitrate to nitrite.*

In addition to the aforementioned distinction of the areas of vanadium involvement in biological systems, it is also interesting to make a classification of the organisms that bind vanadium naturally. At present, six classes of organisms can be recognized on the basis of their ability to biosynthesize a vanadium-containing natural product:

1) A few toadstools of the genus *Amanita*;
2) several families of tunicates;
3) the fan worm *Pseudopotamilla occelata*;
4) the algae, fungus and lichen from which haloperoxidases have been isolated;
5) the bacteria from which the vanadium nitrogenases have been isolated;
6) the bacteria from which the molybdenum-free nitrate reductase enzymes have been isolated.

Many of these species are marine organisms, which is not so strange in view of the abundance of vanadium in the oceans. In this respect, it is important to note that vanadium shows a 'nutrient-like' distribution profile as a function of ocean depth: the depletion in surface ocean water indicates that vanadium is an essential mineral for surface-living organisms.[3] For these reasons, the marine environment is a likely place to find new vanadium proteins or other biological vanadium complexes.[59] The vanadium contents of up to 4% that are found in fossilized marine material, in particular certain oil reservoirs,[60,61] indicate that biogeneous enrichment of vanadium in (marine) organisms is in fact a common phenomenon. The variable $^{50}V/^{51}V$ isotopic ratios that are found in certain petroleum asphaltenes support the proposition that vanadium present in fossilized material is indeed of biological origin.[62]

Impetus for the development of the coordination chemistry of vanadium

The recognition of vanadium as a biometal not only provided a biological impetus. It also fueled fundamental chemical studies of vanadium. Recently, the advances in vanadium chemistry over the past fifteen years were dicussed in a review by Crans.[63] Other important contributions in this respect are from the groups of Rehder, Kiss, Pettersson and Conte. In the fundamental chemical studies of the various systems in which certain biological responses are observed or anticipated, questions are posed such as: 'What species exist in solution?', 'What are the structures of the species in (aqueous/organic) solution?', 'How do the species react?', and 'What are their biological effects?'. In addition to the studies on natural systems and their models, the coordination chemistry of vanadium has also been developed through studies on the synthesis, reactivity and structural aspects of basic vanadium compounds such as vanadate, tris(alkoxide)oxovanadium(V) and bis(acetylacetonato)oxovanadium(IV).[64]

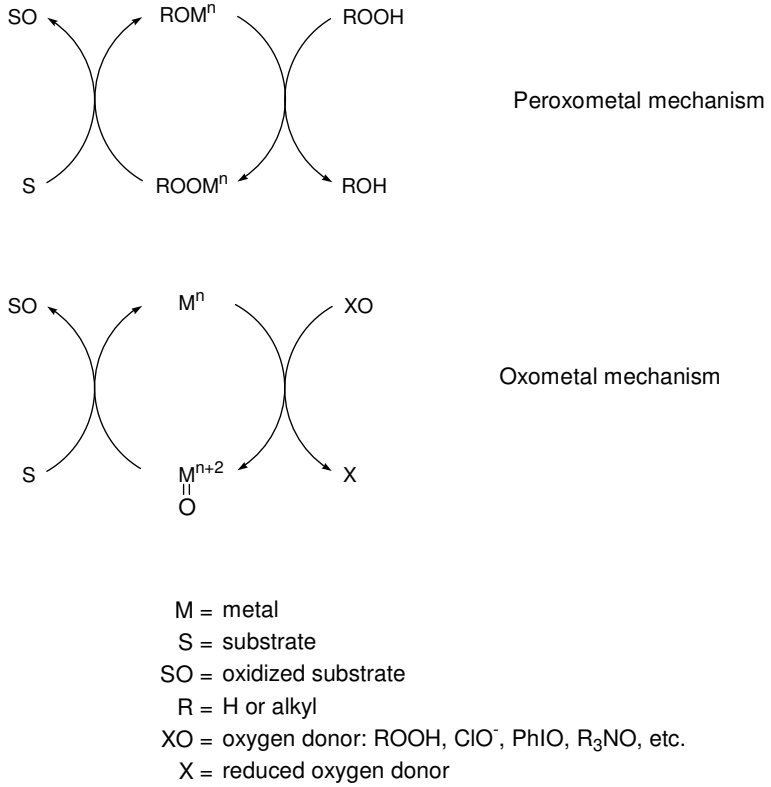
1.3. Vanadium in homogenous catalysis

In the first transition series, vanadium is the last element before some of the (n–1)d electrons begin to enter the inert electron-core of the atom and are therefore not available for bonding.[1] As a result, not only is its melting point the highest in the series but vanadium is also the last element whose compounds in the group oxidation state (i.e. involving all ns and (n–1)d electrons) are not strongly oxidizing. Vanadium's righthand neighbour chromium,[65] for example, has a lower melting point, boiling point and enthalpy of atomization. Also, the most stable oxidation state has dropped to +3, while chromium(VI) is strongly oxidizing. Accordingly, vanadium(V) centres combine a low radius to charge ratio with a relatively weak oxidizing capacity, which makes them particularly suitable as Lewis acid catalysts. Undoubtedly, their most prominent use as such is the activation of peroxidic reagents[66,67] to perform two-electron oxidation reactions. A second application of vanadium centres as Lewis

acid catalysts is the activation of functional groups in organic substrates. The most prominent example of this kind of reaction is the vanadium-catalyzed addition of cyanide to aldehydes and ketones, an area that has developed rapidly in the last decade. The areas of vanadium-catalyzed oxidations and vanadium-catalyzed cyanide additions will be discussed in sections 1.3.1 and 1.3.2, respectively, where emphasis will be on the use of chiral vanadium compounds to perform asymmetric catalysis.

1.3.1. Vanadium-catalyzed oxidations

The mechanisms of metal-catalyzed oxidations[68,69] can be divided into two major categories, involving peroxometal and oxometal species as the reactive intermediate, respectively (Scheme 1.5).

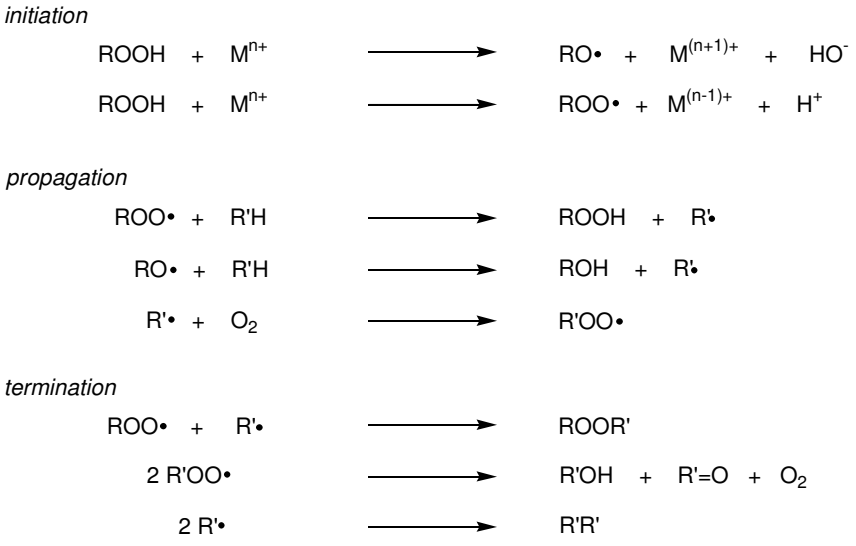


Scheme 1.5. *The peroxometal and oxometal mechanisms in catalytic oxidations.*

Early transition metal catalysts react via the peroxometal mechanism, using a peroxide as the oxygen donor: the metal acts as a Lewis acid to acitivate the peroxide, and the actual oxidant is an intermediate peroxometal species. The oxidation state of the metal does not change during the catalytic cycle. On the other hand, late transition metal catalysts react via the oxometal mechanism, in which the metal cycles between the oxidation states M^n and M^{n+2}, where the latter is present as an oxometal intermediate. In this case, a larger variety of oxygen donors can be used than only peroxides. Vanadium usually operates via the peroxometal mechanism. Only in a few specific cases, vanadium is known to react via a V(III)–V(V) oxometal cycle.

Examples are the oxidation of alcohols by peroxides[70,71] and the oxidation of HBr by a V(III)-tetraethylene glycol pentagonal-bipyrimidal complex.[72]

When performing transition metal catalyzed oxidation reactions, one always has to be aware that metals can induce one-electron oxidation reactions. The reaction involves a radical pathway, in which the metal ion acts as an initiator rather than as a catalyst (Scheme 1.6). In this respect, the two principal reactions of peroxides with metal complexes are oxidation or reduction of the metal under the formation of an oxo- or a peroxo radical (*initiation*). In the presence of organic substrates R'H, the radicals produce organic free radicals (*propagation*), which can undergo dimerization, oxidation or reduction (*termination*).

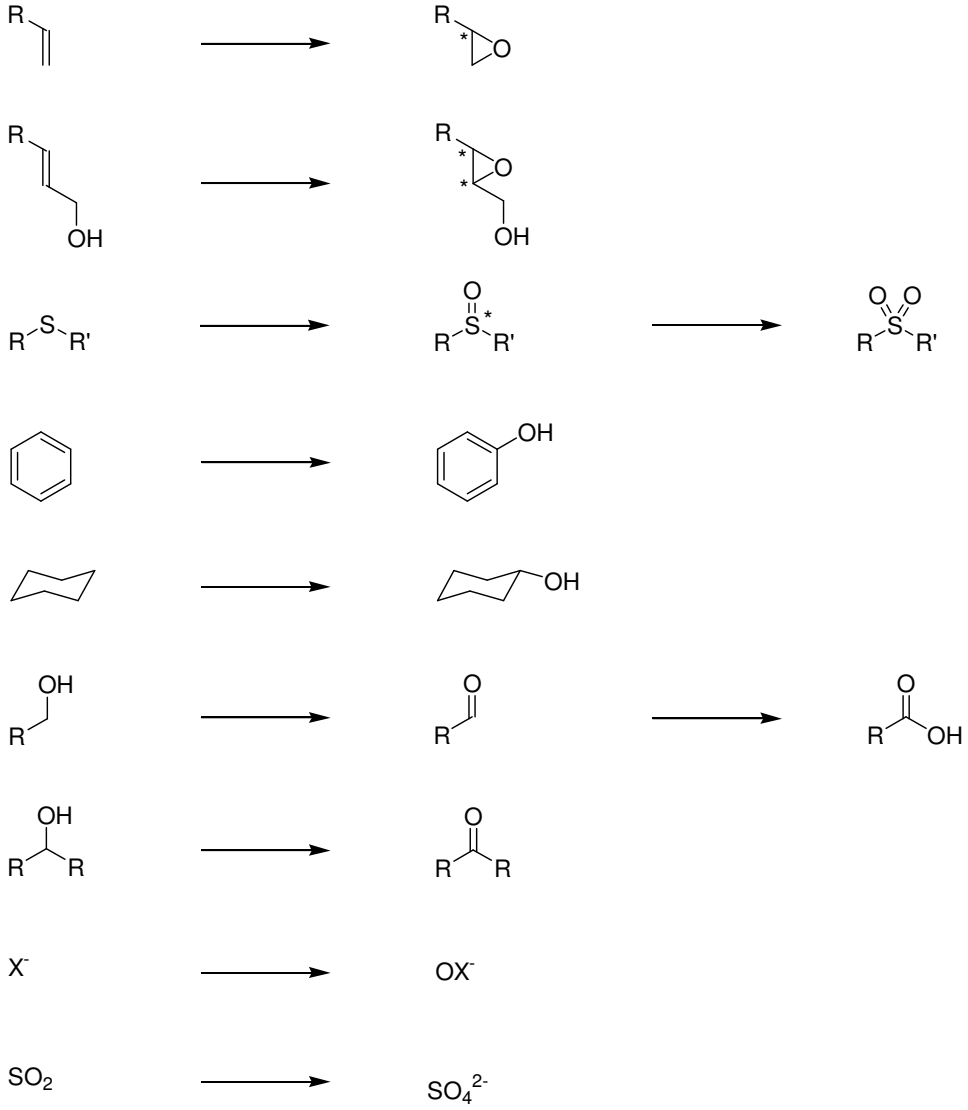


Scheme 1.6. *Oxidation of organic substrates via a radical chain reaction.*

This type of oxidation chemistry is generally referred to as 'Fenton chemistry'. The radicals that are present are strong H-abstractors, which leads to indiscriminate attack on the substrate and unselective reactions; many organic substrates are destroyed rather than converted into desired products, which strongly reduces the applicability of the method. In the presence of hydrogen peroxide or *tert*-butyl hydroperoxide vanadium(IV) and (V) species can induce this chemistry as well.

When designing an oxidation catalyst, for most purposes one must avoid the Fenton chemistry and focus on catalytic processes that proceed via the peroxometal and oxometal mechanisms. Being an early transition metal, vanadium participates via the peroxometal mechanism using peroxides as the oxidants. Vanadium(V) complexes have been found to perform a variety of two-electron oxidation reactions:[73,74,75] 1) alkenes and allylic alcohols can be epoxidized and hydroxylated, 2) sulfides can be oxidized to sulfoxides and sulfones, 3) benzene and other arenes and alkanes can be hydroxylated, 4) primary and secondary alcohols can be oxidized to

aldehydes and ketones via the oxometal mechanism, 5) halides can be oxidized to halogen species that are capable of halogenating a range of organic substrates and 6) sulfur dioxide can be oxidized to sulfate (Scheme 1.7).



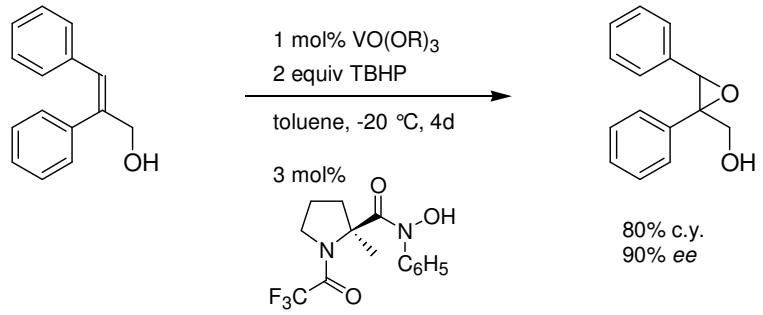
Scheme 1.7. *Examples of the reaction types mediated by vanadium complexes.*

In these reactions, stereogenic centres in the products are created only in the alkene epoxidation and sulfide oxidation reactions.[76] Asymmetric versions of these two oxidation reactions are discussed below.

(1) Vanadium-catalyzed asymmetric epoxidations

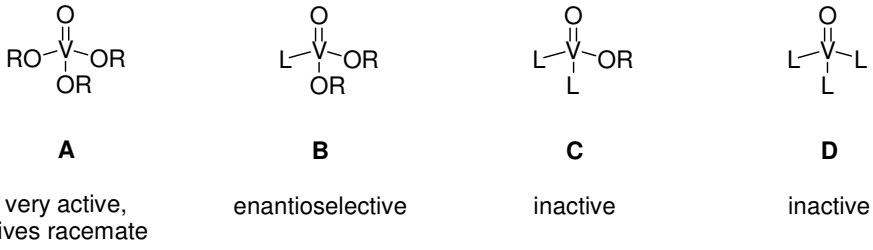
One of the most effective, reliable and practical methods in asymmetric oxidation catalysis is the titanium(IV)-tartrate catalytic system for the asymmetric epoxidation of allylic alcohols with *t*-butyl hydroperoxide, which was described by Sharpless and coworkers.[77,78] Prior to this discovery, they put much effort into the development of vanadium complexes with chiral hydroxamate ligands for the same purpose.[79,80] They reported that these ligands are very

resistent to oxidation and bind well to the metal center. After the exploration of more than twenty chiral hydroxamate ligands for a variety of allylic alcohols, it seemed that an optimum case had been reached. The reaction is then carried out at –20 °C for 4 days, using the combination of two equivalents of TBHP, 1 mol% of vanadium(V) and 3 mol% of chiral ligand (Scheme 1.8).



Scheme 1.8. *Optimized conditions for asymmetric epoxidation as found by Sharpless and coworkers.*

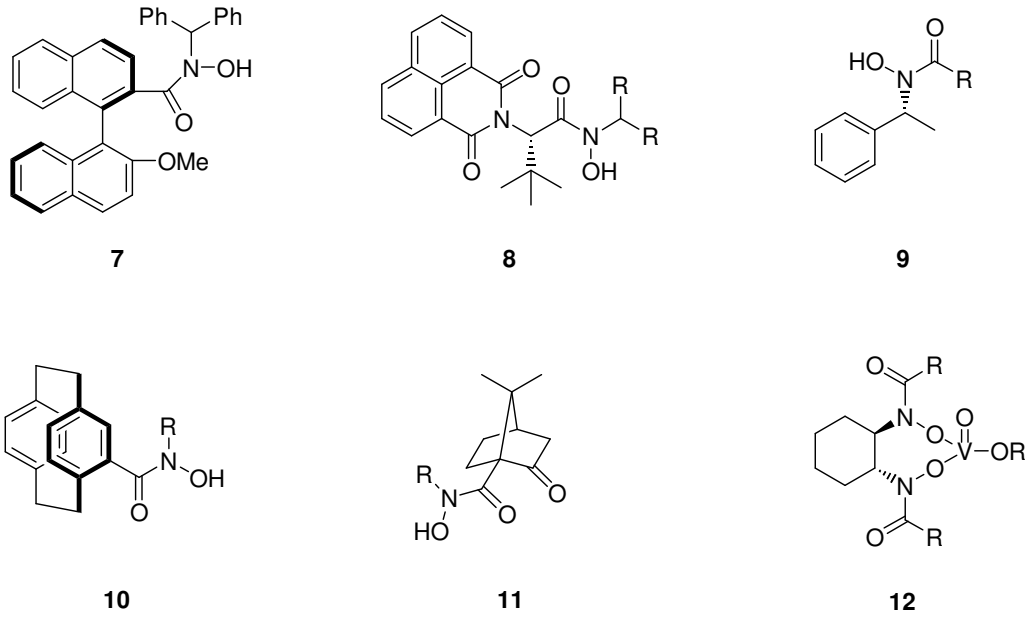
Whereas the vanadium-catalyzed reaction in the absence of hydroxamate ligand is complete in less than one day, the enantioselective reaction as shown in Scheme 1.8 requires four days. Moreover, three equivalents of ligand per vanadium were necessary to obtain a high enantioselectivity. This can be rationalized by assuming the presence of multiple vanadium alkoxide complexes by progressive replacement of the three alkoxide groups[81] (Scheme 1.9).



Scheme 1.9. *Different activities of V(V) alkoxide complexes in epoxidations (L = hydroxamate ligand).*

Due to the rapid ligand exchange in vanadium(V) alkoxides, equilibria exist between the complexes **A**, **B**, **C** and **D**. Which of these is the main species can be tuned by changing the vanadium to ligand ratio, but it will always be accompanied by some of the other alkoxide complexes. To avoid the racemic pathway with complex **A**, all the vanadium must be kept in de inactive forms **C** and **D**. The occurrence of an enantioselective turn-over then depends on the occasional disproportionation of two species **C** into the active catalyst **B** and the inactive form **D**. In this manner, the complete suppression of catalyst **A** slows down the turn-over frequency in favour of a higher enantioslectivity. An optimum case would therefore be a combination of high *ee* values with acceptable reaction times; this appeared to be the case with three equivalents of the chiral ligand per vanadium (Scheme 1.8).

The phenomena described above give a good explanation why the development of this catalytic system to a useful level is so difficult. In 1999, however, Yamamoto and coworkers reported on a significant improvement of the existing protocol,[82] yielding the epoxyalcohols in *ee*'s of up to 94%. The main change in ligand structure is the axial chirality of the ligand (structure **7** in Figure 1.4) and the increased bulk on the hydroxylamine-nitrogen and on the peroxide. Only 1.5 equivalents of the chiral ligand per vanadium were needed, which was interpreted as evidence for the formation of a 1:1 complex between vanadium and the ligand. This finding boosted the investigations on vanadium-hydroxamate catalysed epoxidation of allylic alcohols and several new hydroxamate ligands were explored by Yamamoto[83,84] as well as by other scientists.[76,85] Some of these new ligands are shown in Figure 1.4 (structures **8**–**11**), the R groups representing a large variation of bulky alkyl and aryl groups. Recently, the group of Yamamoto reported on the catalytic action of the vanadium complex of C_2-symmetric bishydroxamate ligand **12**, which for the first time surpasses the titanium(IV) tartrate systems in terms of enantioselectivity.[86]

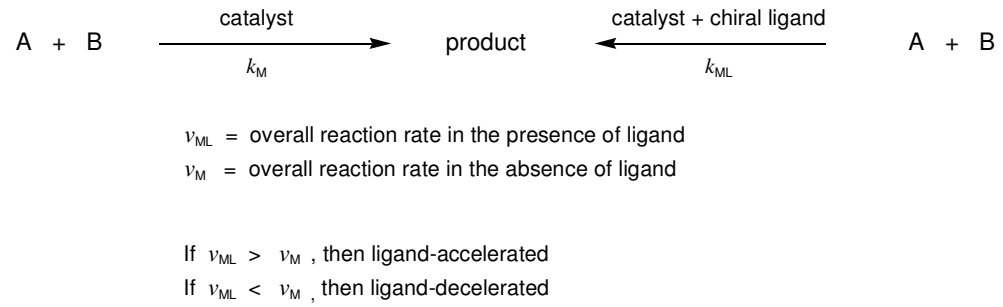


7 **8** **9**

10 **11** **12**

Figure 1.4. *Exploration of hydroxamate ligands since 1999.*

In hindsight, it is recognized that the titanium(IV)-tartrate catalytic system owes its success to the *acceleration* of the reaction by the addition of the tartrate ligand, whereas the catalytic activity of the vanadium is generally inhibited by the addition of the hydroxamate ligand. After the discovery of the phenomenon of ligand-accelerated catalysis (LAC, Scheme 1.10), it was considered that this concept is of general importance for catalyzed reactions to occur in a rapid and highly enantioselective manner,[81] especially for early transition metals where a variety of potent catalytically active species exist in solution due to dynamic ligand exchange processes.

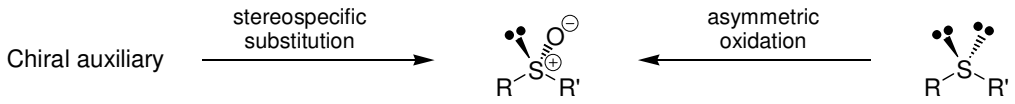


Scheme 1.10. *Ligand acceleration/deceleration.*

Vanadium-catalyzed epoxidation of allylic alcohols with hydroxamate ligands turns into a ligand-accelerated process[87] when it is performed in water, which offers new openings for further research on this catalytic system.

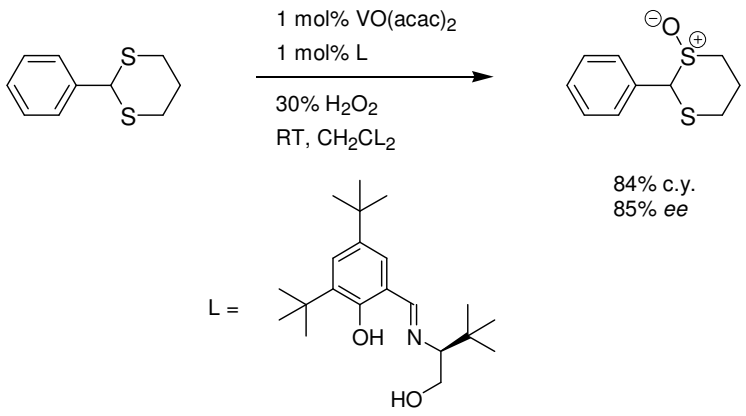
(2) Vanadium-catalyzed asymmetric sulfide oxidations

Chiral sulfoxides are important compounds in asymmetric synthesis, in particular for their use as intermediates in the synthesis of biologically active compounds.[88] A rapidly growing field of research is the application of chiral sulfoxides as chiral auxiliaries or as ligands for catalytic asymmetric transformations.[89,90] The two most common methods for the synthesis of optically active sulfoxides make use of chiral auxiliaries or of metal based catalysts[76] (Scheme 1.11). Among the latter, titanium-based systems are the most prominent, although more recently also vanadium complexes have become an important subject of investigation. It has been found that many vanadium complexes exhibit a high selectivity towards the sulfoxide and produce almost no sulfone: the mechanism of sulfoxide oxidation involves oxygen transfer to the electrophilic sulfur, and the electrophilic character of vanadium-peroxo species results in a decreased catalytic activity in this reaction. This property makes vanadium compounds promising catalysts for asymmetric sulfide oxidations.[75]



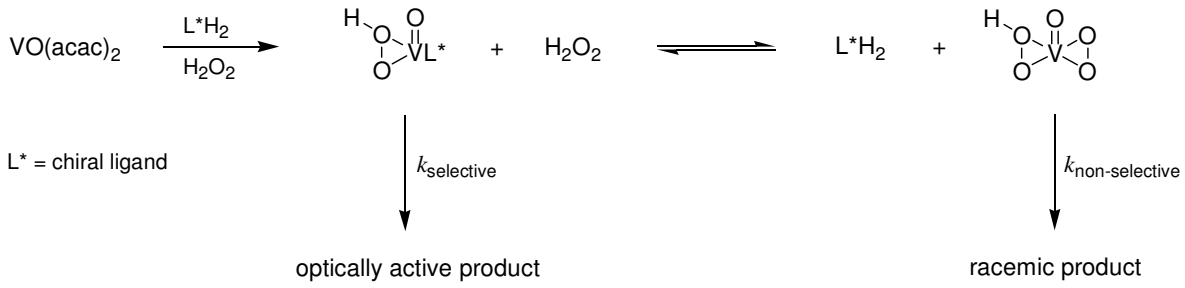
Scheme 1.11. *The two most common routes for the synthesis of optically active sulfoxides.*

In 1995 a very successful vanadium-based system was described by Bolm and Bienewald,[91,92] which has been widely studied since. With aqueous H_2O_2 as the oxidant and readily available vanadium complexes that are prepared *in situ* from $VO(acac)_2$ and a Schiff-base, optically active sulfoxides with *ee*'s of up to 85% were obtained (Scheme 1.12). Remarkable features of this transformation are its efficiency (even with less than 0.01 mol% of catalyst enantioselectivity is achieved) and the fact that it is ligand-accelerated.



Scheme 1.12. *Vanadium-catalyzed asymmetric sulfoxidation with Bolm's Schiff-base system.*

An advantage of the catalyst is the easy variation of its structural and electronic properties, which boosted further exploration of the catalytic system.[76] The simple reaction conditions and the ready availability of the catalyst make the catalytic system attractive for large scale applications, but this is still hampered by the necessity to keep the H_2O_2 concentration at low levels.[93] In the traditional set-up, this is accomplished by using biphasic conditions, but this appears to be a disadvantage when the reaction is performed on a larger scale. Efforts to make the reaction homogeneous by changing the solvent led to a dramatic decrease in *ee*. An initially proposed rationale for this was the formation of a nonselective vanadium diperoxo species at higher peroxide concentrations[93] (Scheme 1.13), which was confirmed later by spectroscopic studies of Blum *et al*.[94]

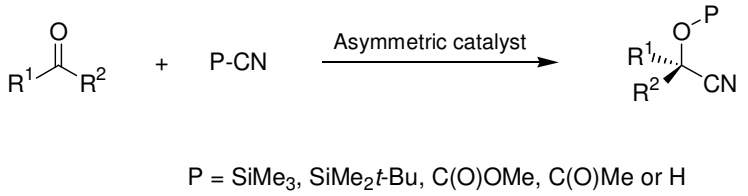


Scheme 1.13. *Model for the observed loss of enantioselectivity at high H_2O_2 concentrations.*

Since the late nineties, it has been observed that haloperoxidase enzymes from several different species are also capable of oxidizing organic sulfides.[95,96,97,98] The mechanism seems to be similar to that of the haloperoxidase action of the enzyme and no redox cycling of the vanadium has been observed. It is also similar to the electrophilic mechanism reported for monomeric vanadium complexes[75] as there is little observed overoxidation to the sulfone product. Sulfoxidation activity has also been observed in a vanadate-substituted phytase from *Aspergillus ficuum*[44] (see also section 1.2).

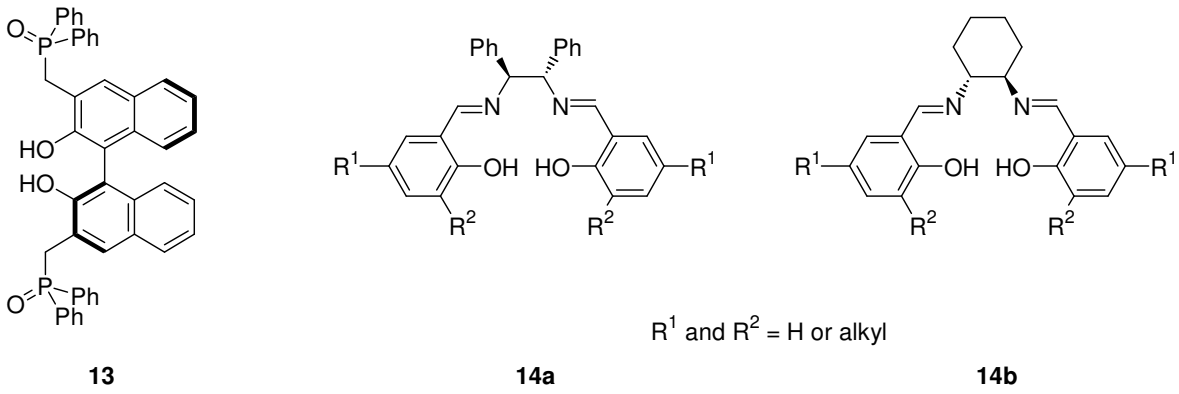
1.3.2. Vanadium-catalyzed cyanide additions

Optically active cyanohydrins are important building blocks in organic synthesis as they may readily be converted into a variety of key functional groups, such as α-hydroxy acids, α-hydroxy esters, α-hydroxy ketones, β-hydroxy amines and α-amino nitriles.[99,100] The key-step of (asymmetric) cyanohydrin synthesis is the addition of cyanide to aldehydes and ketones (Scheme 1.14), which traditionally has mostly been carried out by enzymatic methods, by peptide catalysis, or by using predefined stereocenters to direct the cyanation.[101]



Scheme 1.14. *Cyanide addition as the key-step of cyanohydrin synthesis.*

More recently, Lewis acid-catalyzed cyanation methods were shown to offer viable alternatives, relying on the Lewic acid activation of the carbonyl moiety, around which a chiral environment is created that allows asymmetric addition of cyanide. Whereas Lewis acid-catalyzed reactions are generally among the most numerous and best-studied catalytic reactions,[102,103] a review by North in 1993[104] on catalytic asymmetric cyanohydrin synthesis had just fourteen references on metal-catalyzed systems. Since then, there has been an explosion of interest in the design of metal complexes for the catalytic asymmetric cyanation of aldehydes and ketones.[100,105,106] Various metals and ligands have been explored in different combinations, which has resulted in a number of diverse catalytic systems. Two of the most successful ligands are the bidentate binol (**13**) and the tetradentate salen (**14**) based systems.

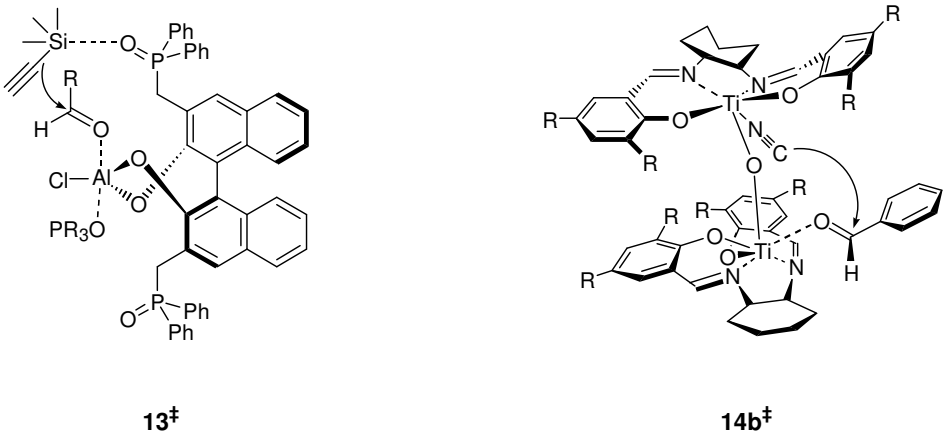


13 **14a** **14b**

Figure 1.5. *Binol (**13**) and salen (**14a** and **14b**) ligands used for catalytic asymmetric cyanohydrin synthesis.*

The exploration of the first was initiated by by Shibasaki *et al.*,[107] while the discovery of the latter system was reported simultaneously by the group of Jiang[108] (**14a**) and the partnership Belokon'/North[109] (**14b**) (Figure 1.5).

The binol system was found to catalyze the asymmetric addition of trimethylsilylcyanide to aldehydes, giving the cyanohydrins in *ee*'s of 83–98%. Best results were obtained when the reaction was carried out at −40°C, using 9 mol% of the catalyst and 36 mol% of an additional phosphine oxide additive under the slow addition of trimethylsilyl cyanide (over 10h). When complexed, the binol species contains both a Lewis acid and a Lewis base moiety and is called a bifunctional catalyst. It is designed to simultaneously bind and activate both the aldehyde (by the Lewis acidic aluminium) and the trimethylsilyl cyanide (by the Lewis basic phosphine oxide). The concept of a bifunctional catalyst can be regarded as an enzyme active site mimic containing Lewis instead of Bronsted acidic/basic sites. Accordingly, bifunctional catalysts are found to drastically improve the efficiency of asymmetric processes with respect to enantioselectivity and/or conversion rate.[110,111] The catalytic cycle proposed by Shibasaki *et al.* involves transition state structure **13**‡ prior to the enantioselecitive intramolecular transfer of cyanide (Figure 1.6).



13‡ **14b‡**

Figure 1.6. *Proposed transition state structures **13**‡ and **14b**‡ of the binol and salen based systems.*

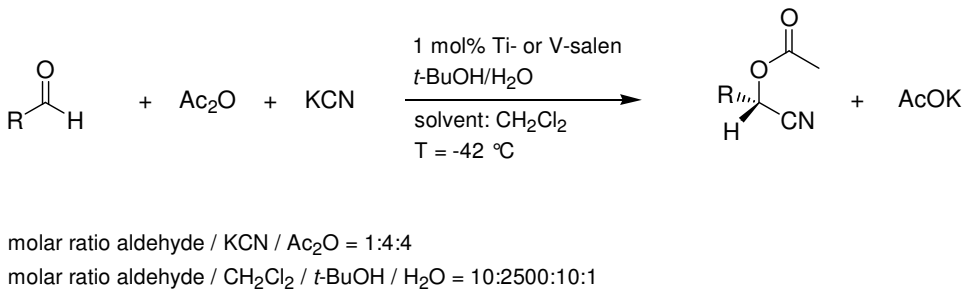
With the use of the tetradentate salen ligands **14** the highest *ee*'s were obtained by Liang and Bu in the case of ligand **14b** bearing $R^1 = R^2$ = *tert*-pentyl. When complexed to titanium tetraisopropoxide, 5 mol% of the resulting complex was used at −78 °C to catalyze the asymmetric addition of trimethylsilyl cyanide to aldehydes, giving the cyanohydrins in *ee*'s of up to 97%.[112] Most studies on the salen based system, however, were performed using ligand **14b** with $R^1 = R^2$ = *tert*-butyl. It was shown by Belokon' and North that the titanium complex of this ligand is an extremely active catalyst: when reactions are carried out at room temperature using 0.1 mol% of catalyst, complete conversion of the aldehyde is reached within one hour in all cases, yielding the corresponding cyanohydrin trimethylsilyl ethers in *ee*'s of up

to 92%. This catalyst was also found to accept some ketones as substrates,[113] which made it the first metal-based catalyst for the asymmetric addition of trimethylsilyl cyanide to ketones at atmospheric pressure. Using ketones as substrates, a larger amount of the catalyst (0.5–1 mol%) was necessary, while the reactions are much slower (1–4 days). Only aryl methyl ketones are effective substrates, allowing substituents at any position of the aromatic ring, including both electron-donating and electron-withdrawing substituents. The corresponding cyanohydrin trimethylsilyl ethers could be obtained in *ee*'s of up to 92%. Further studies by Belokon' and North showed that under the reaction conditions the titanium complex of **14b** is converted into a bimetallic complex having two oxygen bridges.[114] Based on extensive kinetic studies a mechanism was proposed in which the bimetallic complex is the active catalyst, being in equilibrium with the inactive monometallic complex. The active catalyst simultaneously activates both the carbonyl compound and the trimethylsilyl cyanide,[115] which is illustrated by the transition state **14b**‡ in Figure 1.6. Just as in the case of Shibasaki's binol system, the key step is the intramolecular transfer of cyanide to the coordinated aldehyde and the salen system can thus also be classified as a bifunctional catalyst.

After the recognition that the catalyst is extremely active by nature, it was suggested by Belokon' and North that the combination of titanium with the ligand was in fact too reactive. One way of reducing the activity would be to suppress the formation of the catalytically active bimetallic species. To accomplish this, substitution of titanium for vanadium seemed an effective method as titanium(IV)oxo species are generally known to have a greater tendency to form di- or polynuclear species than vanadium(IV)oxo (=vanadyl) species. Indeed, it was shown that when vanadium instead of titanium was used to prepare the catalyst, the reactivity decreased significantly (18 hours to reach complete conversion). Also, for the eight aliphatic and aromatic aldehydes tested, the *ee*'s were 2–25% higher relative to the titanium catalysed reaction.[113,116] It was furthermore shown that the kinetics of the reaction are consistent with the mechanism proposed for the titanium catalysed reaction. The successful vanadium system has drawn the attention of other reserachers with the aim to develop an industrial application of the process in a green context. It has been found that the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate is a good substitute for dichloromethane as a solvent and the ionic liquid catalyst solution is reusable in at least four consecutive runs without losing activity.[117] The same group also reported on the anchoring of the vanadyl salen catalyst to single-wall carbon nanotubes. The catalyst was shown to be truly heterogeneous and could be reused five times; with the asymmetric catalyst an ee of 66% was obtained.[118]

A unique feature of the salen-based catalytic system is the ability of the catalyst to utilise potassium cyanide rather than trimethylsilyl cyanide as the cyanide source (Scheme 1.15).[119] This is beneficial for commercial application of the process since potassium cyanide is

significantly less expensive than trimethylsilyl cyanide and is also less hazardous. For the titanium- as well as the vanadium-catalyzed systems, the yields and *ee*'s obtained with potassium cyanide are similar to the ones with trimethylsilyl cyanide.[120] It appeared that the addition of *tert*-butanol and water to the reaction mixture greatly improved the performance of the system.



Scheme 1.15. *The salen-based catalytic system can utilise potassium cyanide rather than trimethylsilyl cyanide as the cyanide source.*

1.4. Amavadin

1.4.1. Historical background of the research field of trace elements in organic matter

The discovery of the elements of the Periodic System had its most important period in the nineteenth century. Since minerals, ores and other inorganic compounds were the main sources of the elements, studies of their elemental composition received much attention in this period. Also, attention was paid by many chemists to the determination of the composition of natural products. The history of natural product chemistry began in the late eighteenth century, the period in which Lavoisier (1743–1794) discovered that organic compounds contained carbon and hydrogen. One branch of this field concentrated on the presence of trace elements in organic matter. The French biologist and chemist Gabriel Bertrand (1867–1962) made important progress in this area by the determination of many trace elements in living organisms and the specification of their (biological) roles.[121] He introduced the term 'oxidase' for metalloproteins that are capable of catalyzing biological oxidations. In 1894, he put forward the hypothesis that trace elements are co-enzymes necessary for different fermenting systems, which later has been proved justified. In the 1920's, the Dutch chemist Henri ter Meulen (1871–1942) became another important player in the field of trace elements in organic matter.[122] As a Professor in analytical chemistry at the Technische Hoogeschool Delft (now known as Delft University of Technology) he made important contributions to the development of new quantitative analytical methods[123] for the determination of the elements. The idea of collecting certain species from nature to identify their constituents might seem simple and straightforward, but the collected samples were mostly not abundant and the elements to be

determined were present in trace amounts, in contrast to ores and minerals in which these elements were initially found. The analytical methods available at the time had become a serious bottleneck, but with the development of the so-called organic microanalysis in the second decade of the 20th century, many investigations of the presence of trace elements in plants and animals could be initiated. Ter Meulen devoted part of his scientific work to the distribution of molybdenum in nature. He analyzed diverse sorts of organic matter for the presence of this metal,[124,125,47] including leaves, fruits, vegetables, nuts, mushrooms, mineral oils, tissues of man and animals, etc. The largest amount was found in peas (3–9 mg per kg), whereas most of the other samples typically had levels lower by one to two orders of magnitude. A case worth mentioning is the accumulation of molybdenum (1.1 mg per kg) in *Azolla*,[126] a little aquatic plant being abundant in the smaller canals in the neighbourhood of Delft. This report appeared two years after the discovery in 1930 that the bacterium *Azobacter* required molybdenum for nitrogen fixation.[127] It was only decades later that the molybdenum dependent nitrogenase enzymes in these and other bacteria were isolated and characterized.[128]

1.4.2. Discovery of amavadin

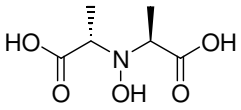
During his investigations on the presence of molybdenum in the mushroom *Amanita muscaria*, Ter Meulen noticed a residual blue solution after precipitation of the MoS_3. Further analysis revealed that vanadium was responsible for this colour and that the mushroom contained 3.3 mg of vanadium per kg.[47]

> *Je fus frappé de la couleur bleue du filtrat obtenu après avoir précipité le MoS_3 de l' Amanita muscaria; je supposai la présence de vanadium ce qui parut être en effet le cas.*

After the discovery of Henze in 1911 that ascidians contain high levels of vanadium,[9] this was the second example of vanadium accumulation in nature. This finding drew the attention of Didier Bertrand (the son of Gabriel Bertrand), who investigated many samples of organic material for the presence of vanadium,[129,130] *Amanita* mushrooms in particular.[48] These studies led to the conclusion that vanadium accumulation was restricted to the mushroom *Amanita muscaria*, which was confirmed in 1978 by studies by Meisch et al[49] on a large number of Middle-European mushrooms. The species *A. muscaria* was found to contain an average of 161 mg of vanadium per kg of dry weight, whereas in all other species the vanadium presence was generally lower by two orders of magnitude. In 1979, however, a few examples of other vanadium-accumulating *Amanita*-mushrooms were reported.[50] The species *A. regalis*, mostly regarded as a variety of *A. muscaria*, was found to accumulate vanadium in average levels of 119 mg/kg of dry weight. Secondly, for *A. velapites* a content of 397 mg/kg of dry weight was found, which surpassed the value of *A. muscaria*. It was furthermore shown

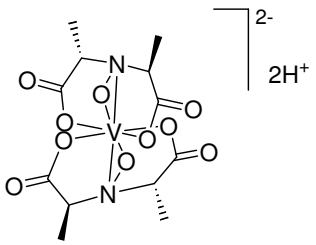
that the measured vanadium contents in *A. muscaria* were not related to the concentration of vanadium in the soil, and that the vanadium is essentially equally distributed between the stem, skin, and cap. Neither was the vanadium content related to the presence of muscarine, a toxin that is present in *A. muscaria*.

In 1972, Kneifel and Bayer[51] were able to isolate a vanadium containing compound from the mushroom *Amanita muscaria*. They named this compound amavadin. The next milestone was the identification of amavadin; the ligand was identified as (2*S*,2'*S*)-*N*-hydroxyimino-2,2'-dipropionic acid **15** (Figure 1.7).[131,132,133] A non-stereoselective synthesis of the ligand was performed[134] involving a complicated separation of the diastereomers,[133] which was followed by the preparation of amavadin. While it was evident that **15** formed a 2:1 complex with vanadium(IV), the structure of amavadin remained uncertain. Initial investigations postulated a V=O species,[131,132] whereas later studies indicated that amavadin was a non-oxo octacoordinated vanadium complex.[135] This was supported by X-ray studies of the related compound V(*N*-hydroxy-iminodiacetate)$_2$[136] and amavadin in its V(V) oxidation state.[137] The coordination sphere of vanadium in these complexes involves four unidentate carboxylate groups and two η^2-NO groups, while the two ligands are connected to the vanadium in a meridional fashion. In 1999 this was confirmed by the group of Garner with X-ray studies on samples of natural amavadin crystallized as phosphoric acid derivative or as the Ca^{2+} salt.[138,139] A schematic representation of the structure of amavadin (**16**) is shown in Figure 1.7. The complex is a divalent acid and is best soluble in water, in which it has excellent hydrolytic stability.



15

ligand of amavadin (LH_3)



16

amavadin ($V^{IV}L_2H_2$)

Figure 1.7. *The structures of the ligand of amavadin (**15**) and the vanadium complex amavadin (**16**).*

Amavadin possesses a reversible one-electron redox couple between the V(IV) and V(V) oxidation states. The potential is remarkably sensitive to solvent; at a Pt electrode it is +0.53 V (*vs* SCE) in water and +0.03 V in DMSO.[140] The biological function of amavadin is still uncertain, but its redox properties have been linked to a possible biological role as a one-electron redox mediator. The observation of the electrocatalytic activity of amavadin in the oxidation of certain thiols suggests that amavadin aids in the self-regeneration of damaged

tissues by cross-linking thiol groups in proteins.[141] Investigations towards the catalytic action of amavadin in oxidation reactions include peroxidative halogenation, hydroxylation, and oxygenation of substrates such as cyclohexane, cyclooctane, benzene and mesitylene at room temperature.[142,143,144,145] Though, these did not give more clarity about a possible biological role of amavadin. It must be stressed that amavadin is not an enzyme nor a co-factor of an enzyme, but that it is a simple and small complex. It is therefore also possible that amavadin is a primitive catalyst which has been substituted by more effective species in the course of evolution. Natural functions that do not involve catalytic action make it more likely that amavadin is essential for the mushroom in these days. An example is the observed ability of amavadin to 'trap' cations, which has resulted in the proposition that the biological function of amavadin involves the selective binding of cations.[146] A more detailed description of amavadin is given in Chapter 4 and Chapter 6 of this thesis, that respectively discuss its structural and catalytic features.

1.5. Research objectives and outline of the thesis

The remarkable structural characteristics of amavadin pose the question as to how far its coordination chemistry can be extended. The main goal of the research described in this thesis was therefore to establish which factors are important for the formation of 'amavadin-type complexes' and to further explore the structural properties of such complexes. The second aim of the research was to apply the vanadium complexes as (asymmetric) catalysts in oxidation reactions and cyanide addition reactions.

An important requirement for the proposed investigations was good access to the optically pure amavadin ligand and to related ligands. Straightforward synthetic strategies were scarcely available, and we started with the development of synthetic routes towards the desired ligands. Once this goal was reached, investigations to the coordination chemistry and catalysis could be performed. Accordingly, the outline of this thesis is as follows:

Chapter 2 describes the first enantioselective synthesis of the amavadin ligand and the preparation of amavadin. Chapter 3 concerns the synthesis of ligands that are related to the amavadin ligand; the new compounds all share the property of being *N*-substituted *N*-hydroxy amino acids. The synthesis and characterization of the vanadium complexes of these ligands is described in Chapter 4. Chapter 5 discusses some chemical and physical properties of amavadin, including its reactivity with peroxides. Finally, Chapter 6 and Chapter 7 deal with the application of amavadin as a catalyst in oxidation reactions and cyanide addition reactions, respectively.

1.6. References

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ENANTIOSELECTIVE SYNTHESIS OF THE AMAVADIN LIGAND AND ITS COMPLEXATION OF VANADIUM

2

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2.1. Introduction

In 1972, Kneifel and Bayer reported the first isolation[1] of amavadin from *Amanita muscaria* and they were also responsible for much of the early work on its characterisation[2] and chemical synthesis.[3,4] After these discoveries, most of the research on amavadin has been focused on its redox properties and structure, while the complexation of transition metals other than vanadium by the amavadin ligand and its derivatives became a second area of investigation.[5] Furthermore, there are some reports on the application of amavadin in catalytic oxidations.[6] Surprisingly, in the reports on the catalytic action no specific attention is paid to the stereochemistry of amavadin and in some cases it is not even clear if optically pure amavadin is used. For a systematic investigation of the catalytic properties of amavadin and for the elucidation of its function in nature, a straightforward synthesis of optically pure amavadin is essential. The only route reported in the literature to date is not stereoselective; after separation of the ligand diastereomers the total yield of amavadin is less than 0.5%.[3,4]

Our first aim was to perform an efficient stereoselective synthesis of the ligand of amavadin, (2*S*,2'*S*)-*N*-hydroxyimino-2,2'-dipropionic acid **1**. As for its complexation of vanadium, the aim was to develop a procedure in which amavadin (**10**; Scheme 2.4) is obtained quantitatively. This requires that the vanadium precursor releases an easily removable molecule after the vanadium complexation by the amavadin ligand. Purification methods such as ion exchange chromatography, which is commonly used for amavadin purification, can then be circumvented. The third aim was to obtain crystals of amavadin in its neutral form **10**, instead of the deprotonated form from which its only X-ray stucture to date has been obtained.

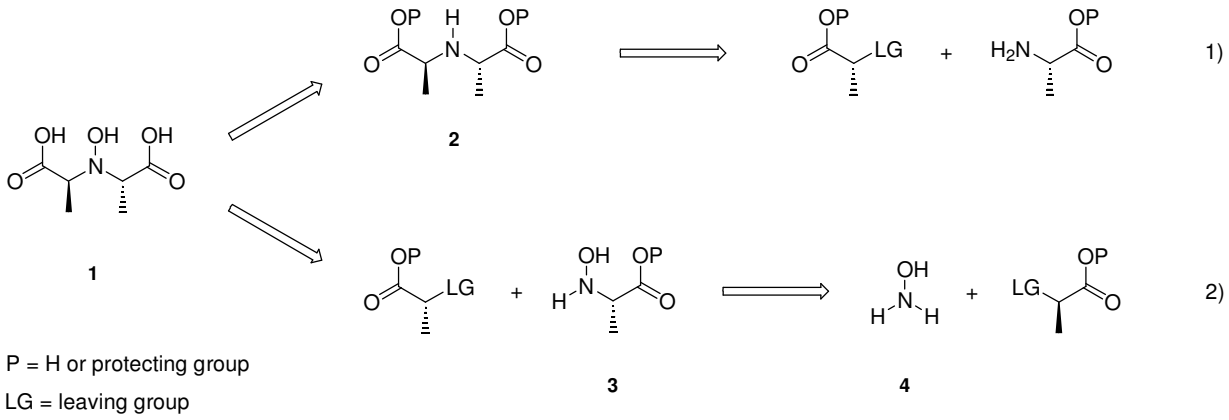
2.2. Enantioselective synthesis of the amavadin ligand

For the synthesis of secondary hydroxylamines like the amavadin ligand **1**, two fundamentally different strategies exist (Scheme 2.1):

1) *N*-oxidation[7] of the secondary amine **2**, which can be prepared from an alanine precursor.
2) Substitutions of hydroxylamine precursors **3** and **4**.

The first strategy has the advantage that a direct and efficient synthesis of the chiral secondary amine **2** has already been described in the literature.[8,9] Assuming that *N*-oxidation does not affect the stereogenic centres, the issue of stereoselectivity is addressed this way. In contrast, the second strategy requires that the stereogenic centres are introduced next to the hydroxylamine function. The synthesis of the amavadin ligand reported in the literature is based on the second strategy: it makes use of the nucleophilic substitution of hydroxylamine **4**

on two equivalents of (*R*)-2-bromo-propionic acid in aqueous solution.[2,10] However, these substitutions do not proceed under complete inversion of configuration and the amavadin ligand was obtained in a maximum *de* of 80%[3] (no *ee* has been reported). After a complicated separation of the stereoisomers, the (*S*,*S*)-isomer was obtained in less than 1% overall yield.[4] In their efforts to synthesize the amavadin ligand in optically pure form, Kneifel and Bayer attempted the *N*-oxidation of **2**, but they were not successful.

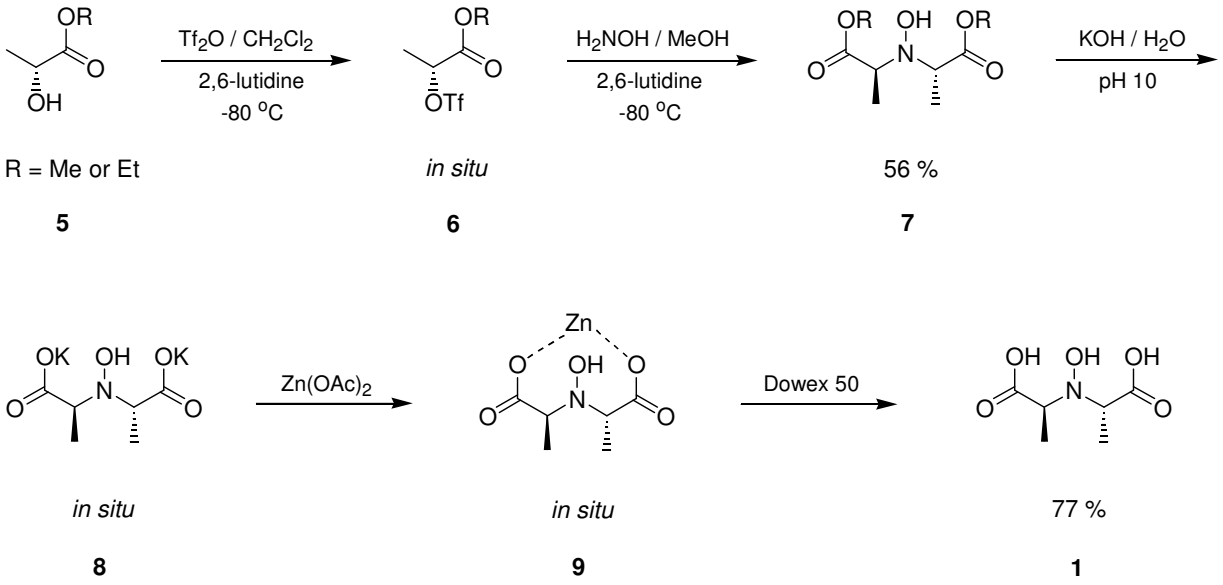


Scheme 2.1. *Retrosynthetic strategy for the synthesis of the amavadin ligand **1**.*

Our investigations towards an efficient stereoselective synthesis were focused on the second strategy. To accomplish a complete inversion of configuration, a leaving group was required that improved the S_N2 character of the reaction. The use of triflate leaving groups for the stereoselective synthesis of the secondary amine **2** was already reported by Effenberger and Burkard.[9] Even more specific is the methodology reported by the group of Ottenheijm,[11,12] in which stereoselective substitutions with hydroxylamines **3** and **4** are performed on triflates. It is furthermore known that selective precipitation of the amavdin ligand with zinc[4] followed by ion exchange chromatography is an effective purification method, because it is very difficult to crystallize the ligand free from cations.[10,13] With the use of these methods, we performed the synthesis of the amavadin ligand in an enantioselective manner.

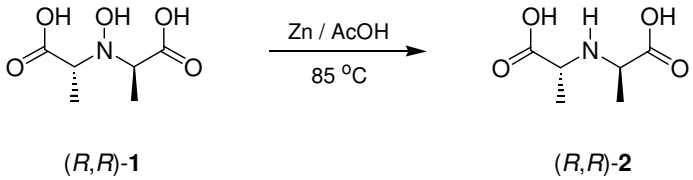
The key-step in our enantioselective synthesis is the nucleophilic substitution on two molecules of *in situ* prepared triflate (**6**) of (*R*)-lactic acid ester (**5**) with hydroxylamine (Scheme 2.2). This proceeds with complete inversion of configuration, yielding 56% of the (*S*,*S*)-*N*-hydroxyimino-2,2'-dipropionic acid ester **7**. Subsequent base hydrolysis[14] under mild conditions yielded the potassium salt **8**, which was purified as its water insoluble zinc-salt **9**. It is important to control the pH carefully during the hydrolysis because substantial decomposition of the ligand takes place at higher pH.[3] Therefore, addition of base with the aid of an automatic burette was necessary. The pure ligand **1** was released by ion-exchange chromatography and isolated, after lyophilization of the aqueous solution, in 77% yield

(starting from **7**). By utilizing (*S*)-lactic acid ester, the (*R*,*R*)-enantiomer of **1** was prepared analogously. In neither of the two syntheses could diastereomers of **7** or **1** be detected, and the optical rotations of the enantiomers of both **7** and **1** had the opposite sign. The optical rotations of the (*R*,*R*)-and the (*S*,*S*)-enantiomer of **1** were $[\alpha]_D^{20}$ = –31.6° and +32.3° (c=1, H_2O), respectively.



Scheme 2.2. *Enantioselective synthesis of the amavadin ligand **1**.*

The presence of **7** as a single enantiomer was furthermore confirmed by chiral GC, and the absolute configuration of **1** was determined by its reduction with zinc to the secondary amine **2**: the (*R*,*R*)-enantiomer of **1** was reduced with retention of configuration to (*R*,*R*)-**2** (Scheme 2.3). This compound is identical to the one described in the literature.[8,9] The measured optical rotation and the literature value are $[\alpha]_D^{11}$ = –11.4° and –11.0° (c=1, H_2O), respectively.



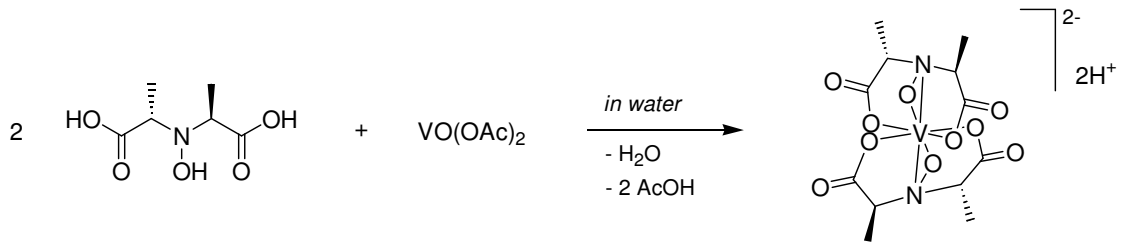
Scheme 2.3. *Reduction of the amavadin ligand to confirm its absolute configuration.*

2.3. Complexation of vanadium by the amavadin ligand

2.3.1. Amavadin in the V(IV) oxidation state

In the earlier reports on the preparation of amavadin,[2,4,10] vanadyl(IV) sulfate ($VOSO_4$) was used as the vanadium source. However, this has significant drawbacks such as the variable water content and the difficult removal of the sulfate, which can both lead to lowered yields. Subsequently, reports on the use of vanadyl(IV)bis(acetylacetonato) (abbreviated as $VO(acac)_2$) appeared, mainly from the group of Garner.[13,15] Furthermore, in one report, Nawi and Riechel[16] used vanadyl(IV)acetate ($VO(OAc)_2$). In vanadium chemistry, $VOSO_4$ and $VO(acac)_2$ are the most frequently used vanadyl(IV) sources, while examples of $VO(OAc)_2$ are rare. In view of the aim to prepare amavadin in a quantitative yield, our investigations focused on the use of $VO(OAc)_2$ and $VO(acac)_2$ as vanadium precursors.

When a suspension of $VO(OAc)_2$ in water was treated with exactly two equivalents of the ligand (LH_3), amavadin (VL_2H_2) was obtained quantitatively after the evaporation of the water and the acetic acid (Scheme 2.4).



1 **10**

Scheme 2.4. *Synthesis of amavadin.*

The analytical data of the synthesized amavadin are identical to the data of amavadin as reported by Kneifel and Bayer.[1,4] The use of $VO(acac)_2$ as the vanadium source in principle gives the same result, although the removal of acacH proved more difficult than that of acetic acid. When the ligand was added to a suspension of $VO(OAc)_2$ in methanol (instead of water), no reaction occurred and the $VO(OAc)_2$ remained in suspension. Only after the evaporation of the methanol and the addition of water amavadin was formed. On the other hand, when homogenous methanolic solutions of $VO(acac)_2$ and the ligand were mixed, the formation of amavadin occurred almost instantaneously.

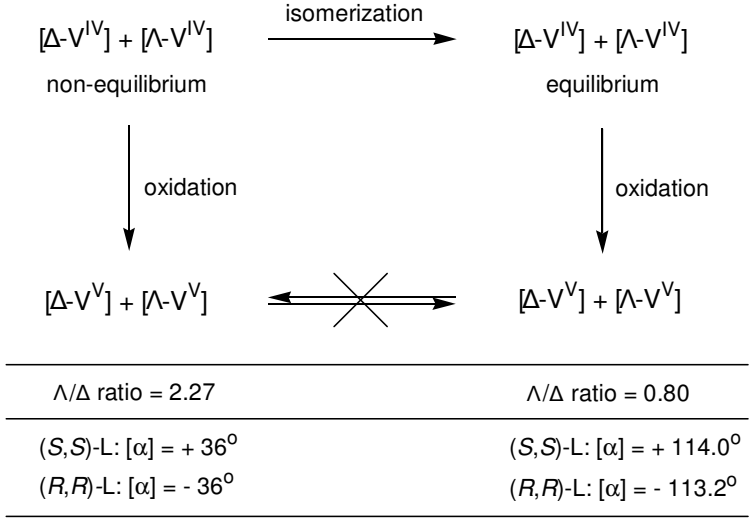
The postulation of Bayer and coworkers in 1987 that amavadin exists as an octa-coordinated vanadium non-oxo compound was confirmed in 1999 by the group of Garner[17] with X-ray studies on samples of natural amavadin. It was crystallized as the Ca^{2+} salt $[\Delta\text{-}VL_2]^{2-}[Ca(H_2O)_5]^{2+}\cdot 2H_2O$ or as the phosphoric acid adduct $[\Lambda\text{-}VL_2H_2]\cdot H_3PO_4\cdot H_2O$, the latter being

solved only at low resolution. Although the exact form in which amavadin is present in the toadstool is not known, it is expected to be deprotonated at physiological pH. Though, from a strictly chemical point of view, the structure of the neutral form of amavadin is also interesting. We attempted to crystallize **10** from water or water/alcohol mixtures, but no solids could be obtained. The only way to obtain solid material was to completely evaporate the solvent, either by rotary-evaporation or by slow evaporation at RT in air. Both yielded blue, glittering solids that were not suitable for an X-ray determination.

2.3.2. Amavadin in the V(V) oxidation state

When amavadin was oxidized with $(NH_4)_2Ce(NO_3)_6$ to the diamagnetic vanadium(V) and isolated as $[VL_2]^-[PPh_4]^+$ (complex **11** in Scheme 2.6), it was possible to carry out NMR studies. These confirmed earlier observations[13] that amavadin exists as a mixture of Δ and Λ diastereomers due to the additional chirality at the vanadium.[18]

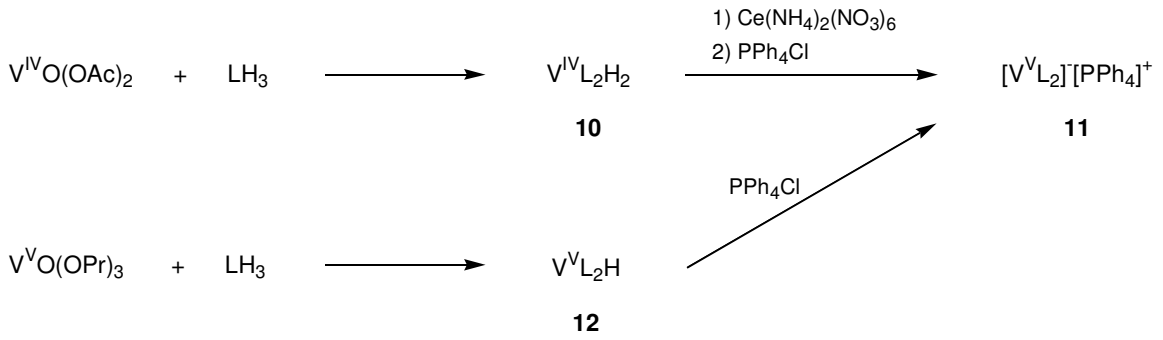
We also observed that the optical rotation of a freshly prepared solution of amavadin changed slowly over a period of several days. This is due to changes in the diastereomeric ratio, which can occur because the vanadium atom is configurationally labile. At several stages of the epimerization process, the ratio of Δ and Λ amavadin in water could be determined via NMR, after oxidation to the vanadium(V) complex. No epimerization was observed after oxidation had taken place. The signals in ^{1}H NMR were clearly separated, which allowed an accurate determination of the isomer ratio (see also Figure 4.5 on p. 94). Directly following its synthesis from $VO(OAc)_2$, the Δ to Λ ratio of amavadin was 2.27 and it decreased to 0.80 after equilibrium had been reached (Scheme 2.5).



Scheme 2.5. *The epimerization of amavadin was followed with ^{1}H NMR and optical rotation measurements.*

In the same period of time, the optical rotation changed from $[\alpha]_D^{25}$ = +36° to +114.0° (c=0.5, H_2O). For amavadin that was prepared from the ligand antipode, the optical rotation changed from $[\alpha]_D^{25}$ = –36° to –113.2° (c=0.5, H_2O).

Furthermore, we found that direct complexation of the vanadium(V) precursor $VO(OPr)_3$ by two equivalents of the amavadin ligand in methanol is a second method to prepare oxidized amavadin. This procedure allowed the $[VL_2]^-$ species to be isolated as $[VL_2]^-[H]^+$ compound **12**, whereas the oxidation procedure using $(NH_4)_2Ce(NO_3)_6$ required extraction with PPh_4^+ ions to afford the $[VL_2]^-[PPh_4]^+$ compound **11**. Compound **12** appeared to be unstable in water,[19] but could be converted into the stable complex **11** after the *in situ* addition of PPh_4Cl (Scheme 2.6).



Scheme 2.6. *Synthesis and isolation of **11**, the vanadium(V) analogue of amavadin.*

2.4. Conclusions

In conclusion, a straightforward stereoselective three step synthesis yielding 43% amavadin was developed. In contrast to the route reported earlier, the synthesis of amavadin can now be performed on a preparative scale. This opens the way to systematic studies of the properties of amavadin, in particular as an asymmetric catalyst. However, these studies also re-emphasize that amavadin is a mixture of two diastereomers, which may limit the achievable enantioselectivity in catalytic reactions.

2.5. Experimental

General considerations: Column chromatography was carried out with silica gel 0.060-0.200 mm, pore diameter approx. 6 nm (Fluka silica gel 60). TLC was performed on 0.20 mm silica gel aluminium sheets (Merck silica gel 60 F_{254}). Elution was carried out with mixtures of petrol ether (PE; boiling point range of 40–65 °C) and ethyl acetate (EtOAc). The plates were developed by dipping in a vanillin bath (15 g of vanillin in 250 mL of ethanol and 2.5 mL of conc. H_2SO_4) where all esters **7** gave orange spots. Ion exchange chromatography was carried out with DOWEX 50WX8-200. ^1H, ^{13}C and ^{51}V NMR

spectra were recorded on a Varian VXR-400S (400 MHz) or a Varian Unity Inova 300 (300 MHz) instrument. Chemical shifts of the ^{1}H and ^{13}C nuclei are expressed in parts per million (δ) relative to tetramethylsilane (in $CDCl_3$) or t-BuOH (in H_2O). Chemical shifts of the ^{51}V nuclei are expressed in parts per million (δ) relative to the external reference $VOCl_3$. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet). Mass spectra were measured on a Micromass Quattro LC-MS spectrometer (Electron Spray Ionization). Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Melting points were measured on a Büchi 510 and are uncorrected. The derivatization of **1** to the dimethyl ester **7** (R=Me) was performed *in situ* with diazomethane, which was generated from *p*-tolylsulfonylmethylnitrosamide (Diazald®). The enantiomeric excesses of **7** were determined by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a 40 m x 0.25 mm chiral column Chiraldex™ B-PH (Beta-cyclodextrin permethylated hydroxypropyl), split injector (1/100) at 220 °C, a Flame Ionisation Detector at 250 °C and using He as carrier gas. Retention times (min) [0 min.: 120 °C; 0–12 min.: 5 °C/min.; 12–20 min.: 180 °C]; (*S*,*S*)-di-methyl ester **7** (5.24), (*R*,*R*)-dimethyl ester **7** (5.45). (*S*)-ethyl lactate[20] and $VO(OAc)_2$[21] were prepared according to standard literature procedures. $VO(acac)_2$ was recrystallized from acetone[22] prior to use. All other chemicals and dry solvents were obtained from Aldrich or Merck and were used as received. Ultrapure water (MilliQ) was used.

Synthesis of *N*-hydroxyimino-(2,2')-dipropionic acid esters (7).

The appropriate lactate **1** was dissolved in 25 mL of dry dichloromethane under a nitrogen atmosphere. At –60 °C, triflic anhydride was added. After 5 minutes, 2,6-lutidine was added and the solution immediately coloured pale red. The mixture was further cooled to –80 °C and during 5 minutes, a solution of H_2NOH in methanol* was added. After the addition the mixture was allowed to warm up to RT. The solvents were removed *in vacuo* at room temperature. The remaining orange oil was purified by flash column chromatography over a 11 x 5 cm silica plug using PE 40-65 / EtOAc = 3 / 2 (for the ethyl ester) or 2 / 3 (for the methyl ester). Compound **7** was then obtained as a yellow oil which crystallized at –18 °C. Recrystallization from PE (ethyl ester) or PE-ether (methyl ester) yielded **7** as white crystals.

* The methanolic H_2NOH solution was prepared as follows: sodium chunks were reacted with 8 mL of methanol under a nitrogen atmosphere. To this solution, an equimolar amount of $H_2NOH{\cdot}HCl$ was added and the mixture was stirred for half an hour. After filtration the solution was ready for use.

(2*R*,2'*R*)-*N*-hydroxyimino-(2,2')-dipropionic acid diethyl ester. From 971 mg (8.22 mmol) of (*S*)-ethyl lactate, 2.589 g (9.18 mmol) of triflic anhydride, 2.045 g (19.08 mmol) of 2,6-lutidine and 263 mg (3.78 mmol) of $H_2NOH{\cdot}HCl$, 491 mg (2.11 mmol, 56%) of **7** was obtained. Mp = 46 °C. $[\alpha]_D^{20} = -9.20°$ (c=2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ; 1.28 (t, 6H, $OCH_2C\underline{H}_3$, J = 7.2 Hz), 1.34 (d, 6H, $NCHC\underline{H}_3$, J = 6.9 Hz), 3.72 (q, 2H, $NC\underline{H}CH_3$, J = 6.8 Hz), 4.22 (q, 4H, $OC\underline{H}_2CH_3$, J = 7.1 Hz), 5.78 (s, 1H, $NO\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ; 172.8 (2C, $\underline{C}OOEt$), 61.0 (4C, $N\underline{C}H$ + $O\underline{C}H_2$), 14.2 (2C, $OCH_2\underline{C}H_3$), 13.0 (2C, $NCH\underline{C}H_3$). MS (*m/z*) 256.5 (ES^+, M+Na).

(2*R*,2'*R*)-*N*-hydroxyimino-(2,2')-dipropionic acid dimethyl ester. From 856 mg (8.22 mmol) of (*S*)-methyl lactate, 2.503 g (8.87 mmol) of triflic anhydride, 1.934 g (18.05 mmol) of 2,6-lutidine, and 257 mg (3.70 mmol) of $H_2NOH{\cdot}HCl$, 208 mg (1.01 mmol, 27%) of **7** was obtained. Mp = 58 °C. $[\alpha]_D^{25}$ = +39.5° (c=2, EtOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ; 1.35 (d, 6H, $CHC\underline{H}_3$, J = 6.9 Hz), 3.74 (q, 2H, $C\underline{H}CH_3$, J = 7.0 Hz), 3.76 (s, 6H, $OC\underline{H}_3$), 5.73 (s, 1H, NO<u>H</u>). ^{13}C NMR (100 MHz, $CDCl_3$) δ; 173.2 (2C, <u>C</u>OOMe), 60.9 (2C, N<u>C</u>H), 52.2 (2C, O<u>Me</u>), 12.7 (2C, $CH\underline{C}H_3$). MS (*m*/*z*) 228.4 (ES^+, M+Na).

(2*S*,2'*S*)-*N*-hydroxyimino-(2,2')-dipropionic acid dimethyl ester. From 1.041 mg (10.00 mmol) of (*R*)-methyl lactate, 3.019 g (10.70 mmol) of triflic anhydride, 2.361 g (22.03 mmol) of 2,6-lutidine and 232 mg (3.33 mmol) of $H_2NOH{\cdot}HCl$, 208 mg (1.01 mmol, 30%) of **7** was obtained. Mp = 58 °C. $[\alpha]_D^{25}$ = –39.2° (c=2, EtOH). ^{1}H NMR, ^{13}C NMR and MS were identical to those of the (*R*,*R*)-enantiomer.

Synthesis of *N*-hydroxyimino-(2,2')-dipropionic acid (1).

In a 100 mL round bottom flask, the diester **7** was dissolved in 10.0 mL of water. An automatic burette was charged with 20 mL of 0.25 M KOH solution. The pH of the reaction mixture was kept at 10.2 by the controlled addition of KOH at RT. After 18 hours the reaction was complete. The pH was brought to 5.5 with diluted HCl and $Zn(OAc)_2{\cdot}2H_2O$ was added to precipitate the ligand. The white precipitate was washed with water and dried *in vacuo*. It was then dissolved in 5 mL of 4 M HCl and charged onto a column of H^+-loaded DOWEX-50 ion exchange resin that was washed with water until neutral pH. Elution with water first gave an eluate with a pH of <1, then the pH increased to 3.8, and when the pH was decreasing again the eluate was collected. Lyophilization of this fraction gave **1** as a white powder.

(2*R*,2'*R*)-*N*-hydroxyimino-(2,2')-dipropionic acid. From 242 mg (1.18 mmol) of (2*R*,2'*R*)-*N*-hydroxyimino-(2,2')-dipropionic acid dimethyl ester **7** and 278 mg (1.27 mmol) of $Zn(OAc)_2{\cdot}2H_2O$, 160 mg (0.90 mmol, 77%) of **1** was obtained. Mp = 144–145 °C. $[\alpha]_D^{20}$ = –31.6° (c=1, H_2O). ^{1}H NMR (300 MHz, H_2O) δ; 1.40 (d, 6H, $CHC\underline{H}_3$, J = 6.9 Hz), 4.0 (q, 2H, $C\underline{H}CH_3$, J = 7.0 Hz). ^{13}C NMR (75 MHz, H_2O) δ; 176.05 (2C, <u>C</u>OOH), 64.29 (2C, <u>C</u>H), 13.15 (2C, $\underline{C}H_3$). MS (*m*/*z*) 178.3 (ES^+, M+1), 176.3 (ES^-, M–1).

(2*S*,2'*S*)-*N*-hydroxyimino-(2,2')-dipropionic acid. From 195 mg (0.95 mmol) of (2*S*,2'*S*)-*N*-hydroxyimino-(2,2')-dipropionic acid dimethyl ester **7** and 220 mg (1.00 mmol) of $Zn(OAc)_2{\cdot}2H_2O$, 61 mg (0.34 mmol, 36%) of **1** was obtained. Mp = 143–144 °C. $[\alpha]_D^{20}$ = + 32.3° (c=1, H_2O). ^{1}H NMR, ^{13}C NMR and MS were identical to those of the (*R*,*R*)-enantiomer.

Synthesis of amavadin.

To a suspension of 55.5 mg (0.30 mmol) of $VO(OAc)_2$ in H_2O was added an aqueous solution of 106.3 mg (0.60 mmol) of **1**. After stirring for 45 minutes, the mixture was clear and dark blue. The smell of acetic acid was noticed. All volatiles were removed by rotary evaporation, and the blue residue was redissolved in 2 mL of water. This procedure was repeated until all acetic acid was removed and the mass of the flask did not change anymore. The yield was 120 mg (0.299 mmol, 99.7%) of a blue solid. The values of the optical rotation were obtained from a solution of amavadin, that was left to stand until the optical rotation had reached a constant value (due to isomerization, the freshly dissolved amavadin slowly changes its optical rotation).

(*OC*-6-1'3)-dihydrogen(bis(di(*S*-1-carboxy-κ*O*-ethyl)-η^2-azanolato)vanadium(IV)) (=amavadin). Mp: decomposes. $[\alpha]_D^{25}$ = +114.0° (c=0.5, H_2O). MS (*m*/*z*) 402.6 (ES^+, M+1), 803.2 (ES^+, 2M+1), 400.6 (ES^-, M–1), 801.3 (ES^-, 2M–1).

(*OC*-6-1'3)-dihydrogen(bis(di(*R*-1-carboxy-κ*O*-ethyl)-η^2-azanolato)vanadium(IV)) (=optical antipode of amavadin). Mp: decomposes. $[\alpha]_D^{25}$ = –113.2° (c=0.5, H_2O). MS (*m*/*z*) 402.6 (ES^+, M+1), 803.3 (ES^+, 2M+1), 400.6 (ES^-, M–1), 801.2 (ES^-, 2M–1).

Oxidation of amavadin to (*OC*-6-1'3)-tetraphenylphosphonium(bis(di(*S*-1-carboxy-κ*O*-ethyl)-η^2-azanolato)vanadium(V)) (= $[VL_2]^-[PPh_4]^+$).

Solutions of amavadin were oxidized at different stages of the epimerization process. The oxidation of a completely epimerized sample of amavadin is described here. To a solution of 50 mg (0.125 mmol) of amavadin in 5 mL of water was added a solution of 72 mg (0.131 mmol) of $(NH_4)_2Ce(NO_3)_6$ in 6 mL of water, after which the mixture immediately turned red. A solution of 47 mg (0.125 mmol) of $P(Ph)_4Cl$ in 10 mL of CH_2Cl_2 was added. The mixture was stirred for 15 minutes and the CH_2Cl_2-layer was separated, dried over $CaCl_2$, filtered and evaporated to dryness. Two isomers were formed in a ratio of A : B = 8 : 10. The assignment of the ^1H and ^{13}C resonances was based on this ratio. ^1H NMR (300 MHz, $CDCl_3$) δ; 1.51^B, 1.55^A (d, 6H, CHC$\underline{H}_3$, J = 7.0 Hz), 1.86^A, 1.92^B (d, 6H, CHC$\underline{H}_3$, J = 7.5 Hz), 4.45^A, 4.55^B (q, 2H, C$\underline{H}$CH$_3$, J = 7.5 Hz), 4.84^B, 5.07^A (q, 2H, C$\underline{H}$CH$_3$, J = 7.0 Hz), 7.64^{A+B}, 7.82 $^{A+B}$, 7.94 $^{A+B}$ (m, 20H, P($C_6\underline{H}_5$)$_4$. ^{13}C NMR (75 MHz, $CDCl_3$) δ; 14.09^B, 14.86^A, 15.83^B, 16.07^A (4C, CH$\underline{C}$H$_3$), 66.87^B, 68.42^A, 70.69^A, 71.89^B (4C, $\underline{C}$HCH$_3$), 117.47^{A+B} (d, 4C, P-$\underline{C}$, $^1J_{P\text{-}C}$ = 90 Hz), 130.84^{A+B} (8C, P($\underline{C}_6H_5$)$_4$), 134.42^{A+B} (8C, P($\underline{C}_6H_5$)$_4$), 135.89^{A+B} (4C, P($\underline{C}_6H_5$)$_4$) 174.74^A, 174.33^B, 172.76^B, 172.24^A, (4C, $\underline{C}$OOH). ^{51}V NMR (105 MHz, $CDCl_3$) δ; –290. MS (*m*/*z*) 399.4 (ES^-, M–PPh_4), 738.2 (ES^-, M), 339.6 (ES^+, PPh_4).

Reduction of the (*R*,*R*)-amavadin ligand (1) to (2*R*,2'*R*)-imino-2,2'-dipropionic acid (2).

To a solution of 119 mg (0.67 mmol) of (*R*,*R*)-**1** in 7 mL of 2 M HCl was added 470 mg (7.19 mmol) of Zn-dust. The mixture was heated to 85 °C for 1.75 hour, after which the solution was clear and colorless with a few zinc grains. The pH was brought to 5.5 with 5 M KOH; a white precipitate formed already at pH 2.0. After centrifugation and decanting the supernatant, a white solid was obtained. This solid was dissolved in 5 mL 2 M HCl and was brought onto a column of H^+-loaded DOWEX-50 ion exchange resin that was washed with water until neutral pH. Elution with water first gave an eluate with a pH of <1, after which the pH increased to 4.7. When the pH dropped again, the product fractions were collected. After concentrating the product solution by rotary evaporation followed by lyophilization, 64 mg (0.40 mmol, 59%) of a white solid was obtained. Mp = 240 °C (lit. 242 °C). $[\alpha]_D^{11}$ = –11.4° (c=1, H_2O) [lit: $[\alpha]_D^{11}$ = –11.0° (c=1, H_2O)]. ^1H NMR (300 MHz, H_2O) δ; 1.52 (d, 6H, CHC$\underline{H}_3$, J = 7.50 Hz), 3.89 (q, 2H, C$\underline{H}$CH$_3$, J = 7.20 Hz). ^{13}C NMR (100 MHz, H_2O) δ; 175.05 (2C, $\underline{C}$OOH), 57.38 (2C, $\underline{C}$HCH$_3$), 16.66 (2C, CH$\underline{C}$H$_3$). MS (*m*/*z*) 162.1 (ES^+, M+1), 160.2 (ES^-, M–1).

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SYNTHETIC ROUTES TO AMAVADIN-BASED LIGANDS

3

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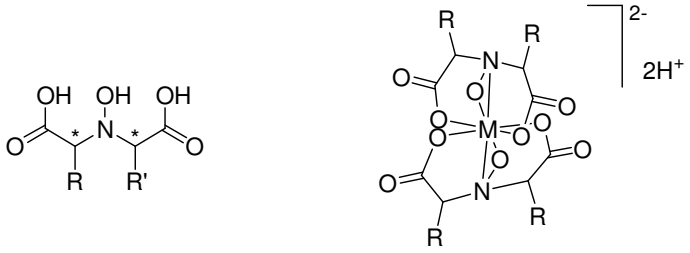
3.1. Introduction

The chemical synthesis of amavadin[1,2] and the investigations of its electrochemistry,[3] catalytic properties[4,5,6,7] and structure[8] have been reported in the literature by others and us. The modifications of amavadin that have been described concern the substituents on the ligand backbone and the complexing metal (Figure 3.1). In most cases, the natural ligand **1** or its simplified achiral analogue **2** was complexed to the early transition metals Ti, Zr, Nb, Ta and Mo. In a particular case, a ligand with ethyl groups on the backbone was prepared (**3**) and its complexation with V and Mo was studied. A review on the reported metal complexes of ligands **1**–**3** is presented in Chapter 4.

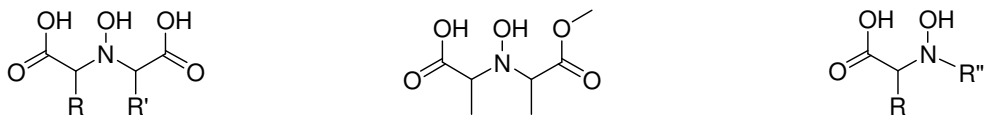


1: R = R' = Me
2: R = R' = H
3: R = R' = Et

Figure 3.1. *Amavadin analogues reported in the literature.*

3.1.1. Aim

A remarkable feature of amavadin is that it is an air-stable non-oxo vanadium(IV) complex in which the two *N*-hydroxy groups act as η^2-ligands. It has previously been shown that the modified ligands **2** and **3** do not affect the basic structure of amavadin,[9,10] but that the *N*-hydroxy group is essential for the formation of the non-oxo vanadium(IV) nucleus.[11,12] In order to establish which other factors are important, we focused on the role of the carboxylate groups and on the role of large substituents on the backbone.



4 **5** **6**

R = Ph, Bn, *iso*-alkyl
R' = Me or H

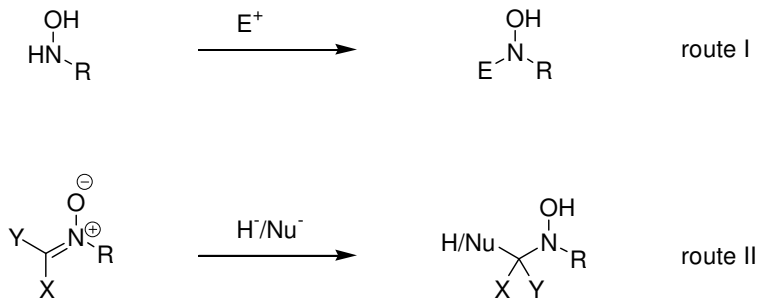
R and R" = carbon substituent without heteroatoms

Figure 3.2. *Amavadin-based ligands **4**, **5** and **6**.*

For this purpose, we aimed to synthesize ligands **4**, **5** and **6** (Figure 3.2), in which one backbone methyl group of **1** is replaced by a larger substituent such as Ph, Bn or *iso*-alkyl (**4**); or in which one coordinating group of **1** is replaced by a weaker coordinating center (**5**) or by a non-coordinating alkyl group (**6**). The synthesis of amavadin-based ligands **4**, **5** and **6** has no precedent in the literature, although a few reports exist on the synthesis of other *N*-alkylated *N*-hydroxy amino acids.[13,14,15,16] The complexation of these ligands to vanadium will be described in Chapter 4.

3.1.2. Strategy

For the synthesis of hydroxylamines, it is instructive to examine the amine counterpart, which is a much more abundant structural element in chemistry. The two most common synthetic routes towards secondary amines are *N*-alkylation of primary amines[17] and additions to imines/imine reduction.[17,18] For the synthesis of secondary hydroxylamines, equivalents for these two strategies exist: the *N*-alkylation of primary hydroxylamines[17,19] and additions to nitrones/nitrone reduction[17,18,20,21] (routes I and II in Scheme 3.1, respectively).



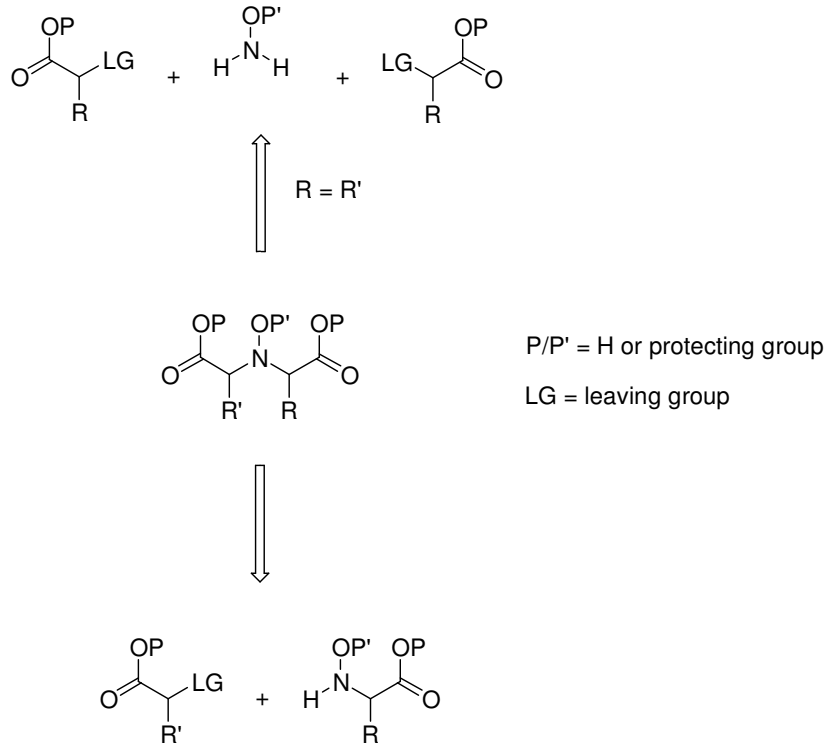
Scheme 3.1. *Two of the main synthetic routes towards secondary hydroxylamines.*

The application of these strategies to the synthesis of secondary hydroxylamines, however, is less general than for the secondary amines. The use of nitrones is generally considered to be the most versatile method of the two. The syntheses of the amavadin-based ligands that are described in this chapter are either based on route I (section 3.2) or on route II (section 3.3). In both routes, *N*-hydroxy amino acid esters are important intermediates. An overview of their syntheses is described separately in section 3.4.

3.2. *N*-alkylation of hydroxylamines

This is a useful method for the synthesis of *N*-hydroxy-α-amino acids and derivatives thereof.[16,19,22,23,24] In the case of the amavadin ligand,[1,2] the *N*-hydroxy compound is either hydroxylamine (H_2NOH) or an *N*-hydroxy amino acid derivative, both with the possibility of

being protected on the hydroxylamine-oxygen. The following synthetic strategy can be formulated to prepare amavadin ligand analogues with variations of R and R' (Scheme 3.2).



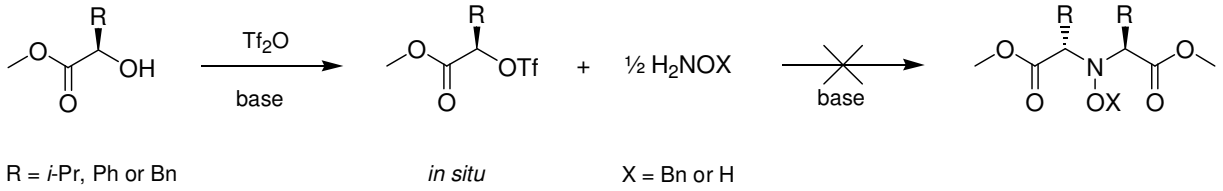
Scheme 3.2. *Retrosynthetic strategy based on N-alkylation of hydroxylamines.*

There are two promising options for the application of this strategy. The first is the use of triflate as a leaving group. The success obtained with the α-triflate esters as described in Chapter 2[2] points to a potentially successful stereoselective application of this method here (section 3.2.1). The second option is the use of bromide as a leaving group. The non-stereoselective literature route to amavadin makes use of α-bromo acids and has proven itself for two analogues (section 3.2.2). For both leaving groups the equivalent reactions yielding the secondary amines are described in the literature,[25,26,27] where the highest stereoselectivities were obtained when triflates were used.

3.2.1. Substitutions on α-triflate esters

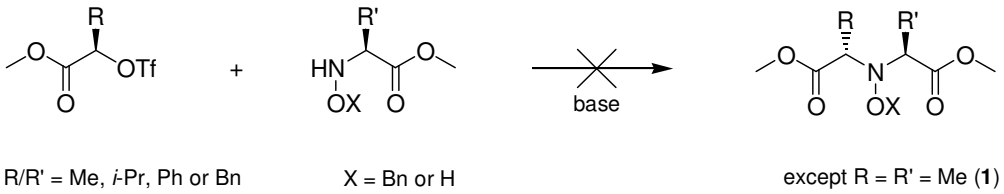
The double substitution of hydroxylamine was attempted in one step using α-triflate esters having an *i*-Pr, Ph or Bn substituent at the R-position (Scheme 3.3), but the application of the exact protocol as described in Chapter 2 did not give the desired product. A first modification was to use pure *O*-benzyl hydroxylamine instead of the *in situ* prepared unprotected hydroxylamine, which allowed the reaction to be carried out in the absence of methanol and NaCl (both hydroxylamines require removal of HCl prior to application, but only the former can be isolated in pure form). Other modifications that were attempted included the use of

different bases (pyridine, triethylamine, imidazole) and a variation of the solvent(s) (methanol/hexane, dichloromethane, DMSO). Unfortunately, neither gave the desired products. In most cases the mono-substituted product was obtained while (part of) the α-triflate ester remained unchanged.



Scheme 3.3. *Attempted double substitutions of hydroxylamine on α-triflate esters.*

In a different approach the reaction was conducted sequentially by performing the synthesis of the *N*-hydroxy amino acid ester and then allowing it to react with triflate. This proved successful for the reaction of *N*-hydroxy alanine ester with the triflate of ethyl lactate, leading – not surprisingly – to the parent structure. However, other *N*-hydroxy esters appeared to react very poorly with the triflate, just as *N*-hydroxy alanine ester with other triflates (Scheme 3.4). The desired products could not be detected and in most cases the starting materials were recovered, confirming lack of reactivity which was also observed in the attempts shown in Scheme 3.3.



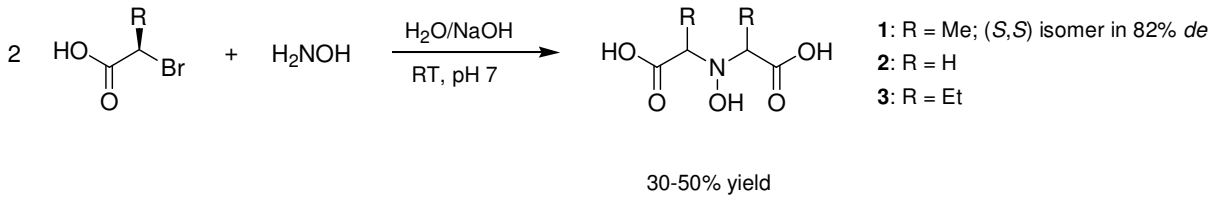
Scheme 3.4. *Attempted substitutions of N-hydroxy amino acid esters on α-triflate esters.*

In cases where the triflate of ethyl mandelate was used, the corresponding α-chloro and α-methoxy compounds were isolated, indicating competition between the *N*-hydroxy compound, methanol and chloride as a nucleophile. We attempted to carry out the reaction in the absence of methanol and chloride but their presence could not be avoided after the liberation of H_2NOH from H_2NOH•HCl.

3.2.2. Substitutions on α-bromo acids

The literature reports on the syntheses of the amavadin ligand **1** and its two derivatives **2** and **3** all follow the path of substitution of hydroxylamine on two molecules of α-bromo carboxylic acid in aqueous solution. With this method, the compounds have been prepared in 10 gram

amounts in yields typically ranging from 30% to 50% (Scheme 3.5).[28,29,30] Whereas substitutions on α-triflate esters tend to occur with a high degree of stereoselectivity, the α-bromocarboxylic acids show moderate stereoselectivity. Starting from (*R*)-2-bromopropionic acid, the natural ligand **1** has been prepared in 82% *de*[29] (the literature does not give clarity about the *ee* of the product). The reports furthermore state that it is important to maintain conditions of neutral pH and RT to prevent decomposition of the product and to obtain higher stereoselectivity.



Scheme 3.5. *Literature syntheses of the amavadin ligand and its two derivatives.*

We repeated the syntheses of **1** and **2** and were able to isolate both compounds in yields comparable to those reported in the literature, while the *de* of **1** was 60% (determined by NMR). The optical rotation of (*S*,*S*)-**1** was $[\alpha]_D^{20}$ = +21.3° (c=1, H_2O), while the literature value for **1** obtained via this method is $[\alpha]_D^{20}$ = +25.8° (c=1, H_2O).[29,31] In the next step, this method was applied using α-bromo acids having an *i*-Pr, Ph or Bn substituent at the α-position. However, in no case was any reaction observed at pH 7.2 and the starting material could be re-isolated in high yield. When the pH or the temperature was increased reaction did occur, but only the corresponding α-hydroxy acid could be isolated (in an almost quantitative amount).

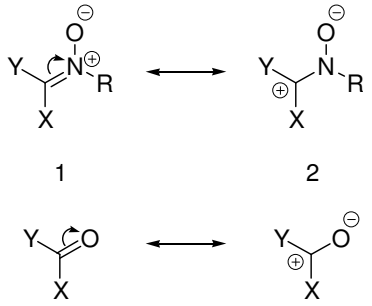
3.3.3. Evaluation of the N-alkylation route

Despite the fact that a certain degree of generality is assigned to *N*-alkylation of hydroxylamines,[17,19] especially in cases where *O*-benzylhydroxylamine is applied,[32] the results in this section show that the alkylation method is of limited applicability for the synthesis of amavadin ligand analogues. The underlying explanation for the low reactivity of hydroxylamines as compared to amines is the inductive effect of the hydroxy group on the nitrogen, which results in a decreased nucleophilicity of the nitrogen. Both with the α-triflate esters and the α-bromo acids we saw that no reaction took place in the case of more bulky substituents, indicating that steric factors also play a role. Because the low reactivity is an intrinsic property of hydroxylamines, the *N*-alkylation strategy was abandoned. A synthetic route that makes use of nitrones was considered to have more perspective for a general route towards amavadin-based ligands.

3.3. Nitrones as precursors for secondary hydroxylamines

3.3.1. Introduction to nitrones[20,21,33,34]

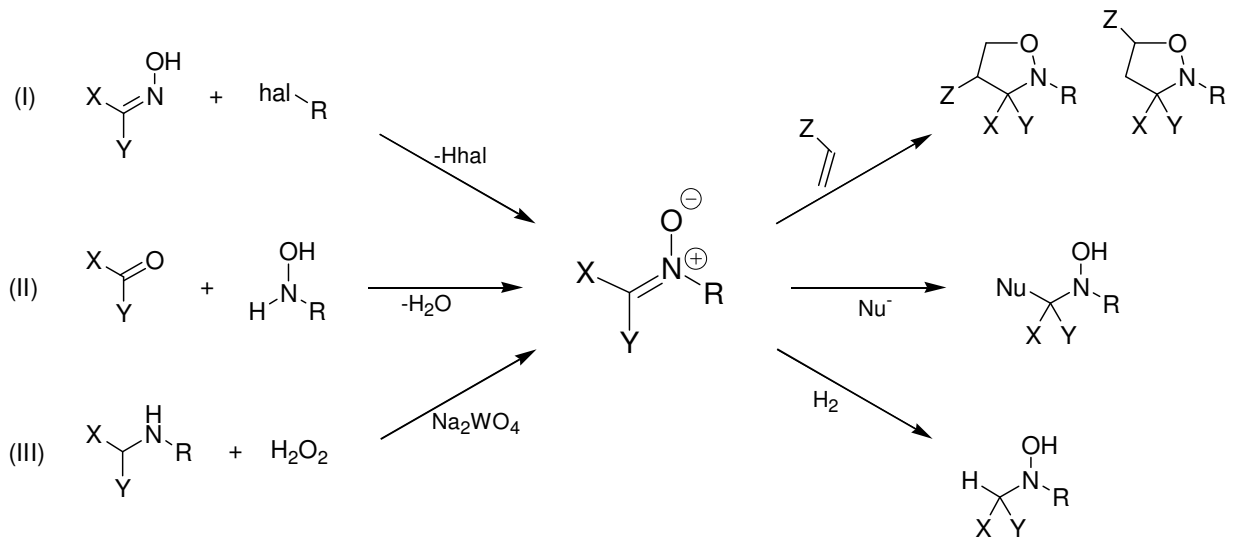
The name 'nitrone' was introduced by Pfeiffer in 1916[35] for compounds possessing the *N*-alkylidene-*N*-oxide or azomethine *N*-oxide group. It is a contraction of 'nitrogen ketone', emphasizing their striking similarity with ketones. The analogy rests on the mesomeric effects, which make the nitrone group behave as an extended carbonyl function (Scheme 3.6). Generally, the term 'nitrone' is restricted to compounds in which the mesomeric forms 1 and 2 contribute most to the structure, which excludes all *N*-oxides in which there is a considerable delocalisation of the positive charge (*e.g.* in pyridine *N*-oxide).



Scheme 3.6. *The similarity of nitrones and ketones rests on their mesomeric effects.*

Nitrones are moderately stable compounds. Especially under the action of light and heat nitrones can rearrange into oxaziridines, secondary amides and/or oxime ethers. Furthermore, nitrones are prone to dimerization, especially α-aliphatic nitrones. It is proposed that after spontaneous enolization of a first nitrone, a cycloaddition reaction to the second nitrone takes place.

The three most important methods to prepare nitrones are depicted in Scheme 3.7. Route (I) comprises the *N*-alkylation of an oxime. This looks like a convenient route, but its practical applicability is rather limited because the oxime is an ambident nucleophile that favours *O*-alkylation in most cases.[36] The most important synthesis route to nitrones[37] is route (II): the condensation of a primary *N*-hydroxy compound with an aldehyde or ketone. This method is of general utility but it depends on the availability of the appropriate *N*-hydroxy compound. Route (III) is the catalytic oxidation of a secondary amine with hydrogen peroxide,[38] which is in fact the most simple and effective method. However, the poor regioselectivity limits its application[39] to symmetric secondary amines[40] and to amines with specific α-carbon substituents that ensure high regioselectivity.[41] Another route that can be used to prepare nitrones is the oxidation of a secondary hydroxylamine, but that would be counterproductive, considering that the aim is to prepare secondary hydroxylamines from nitrones.



Scheme 3.7. *Synthesis and application of nitrones.*

The dipole of nitrones makes them reactive intermediates that have become very important in organic synthesis.[42] They are known to undergo two fundamental reactions[43]: 1,3-dipolar cycloaddition reactions with alkenes and the addition of nucleophiles (Scheme 3.7). The latter reaction is relevant to the development of routes towards new amavadin ligands, as it produces secondary hydroxylamines and allows variation of the substituents on the α-carbon atoms. The addition can be performed using nucleophiles such as cyanide and carbanions (formation of new C-C bonds), but can also be accomplished with reducing agents such as $LiAlH_4$, $NaBH_4$, and $NaCNBH_3$. These nitrone reductions usually stop at the hydroxylamine stage.

Based on these reactions, we formulated the following three synthetic strategies (Scheme 3.8):

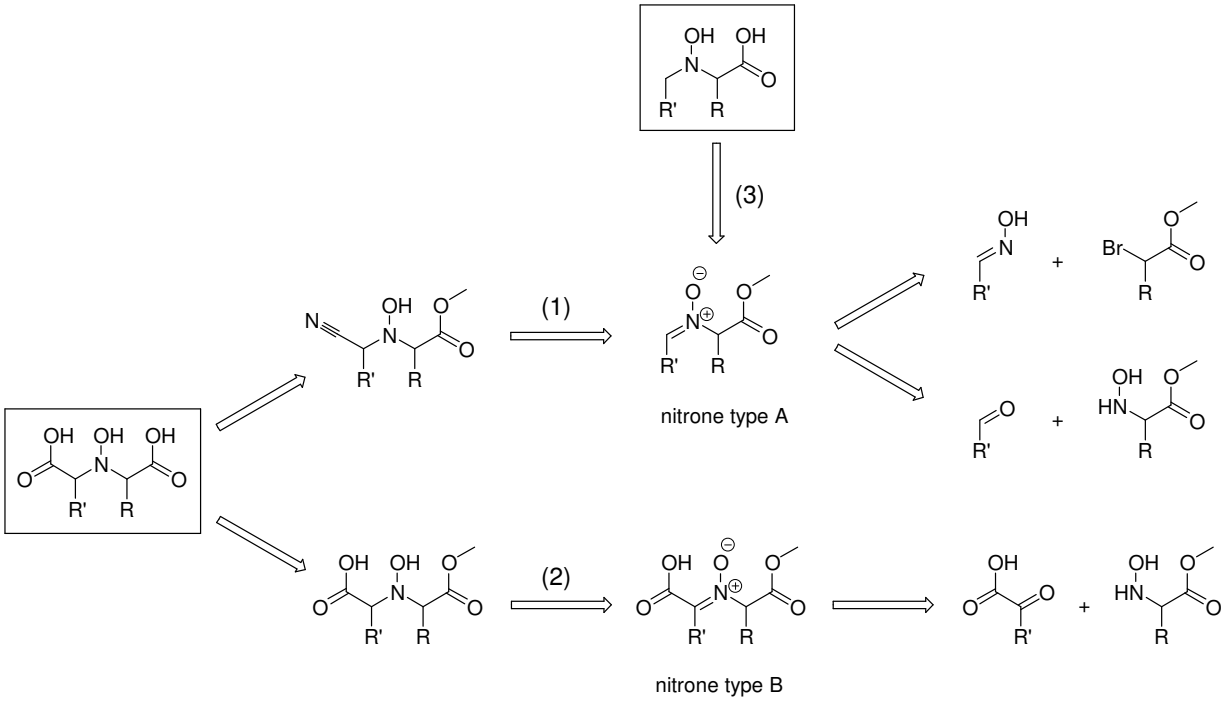
Nitrone cyanation (3.3.2):

(1) Addition of cyanide to nitrones of type A, followed by nitrile and ester hydrolysis. The cyanation of nitrones is well-documented in the literature[44,45,46] The hydrolysis of the resulting α-cyano secondary hydroxylamines is considered a powerful route towards *N*-substituted-*N*-hydroxy amino acids,[38] although the literature gives only few examples.[47,44]

Nitrone reduction (3.3.3):

(2) Reduction of nitrones of type B, followed by ester hydrolysis. The reduction of nitrones[48,49,50,51] is described less frequently than the cyanation of nitrones, however asymmetric versions have been reported.[52,53,38]

(3) Reduction of nitrones of type A, followed by ester hydrolysis. The target products are *N*-alkylated *N*-hydroxy amino acids, which have not been prepared before in this way.



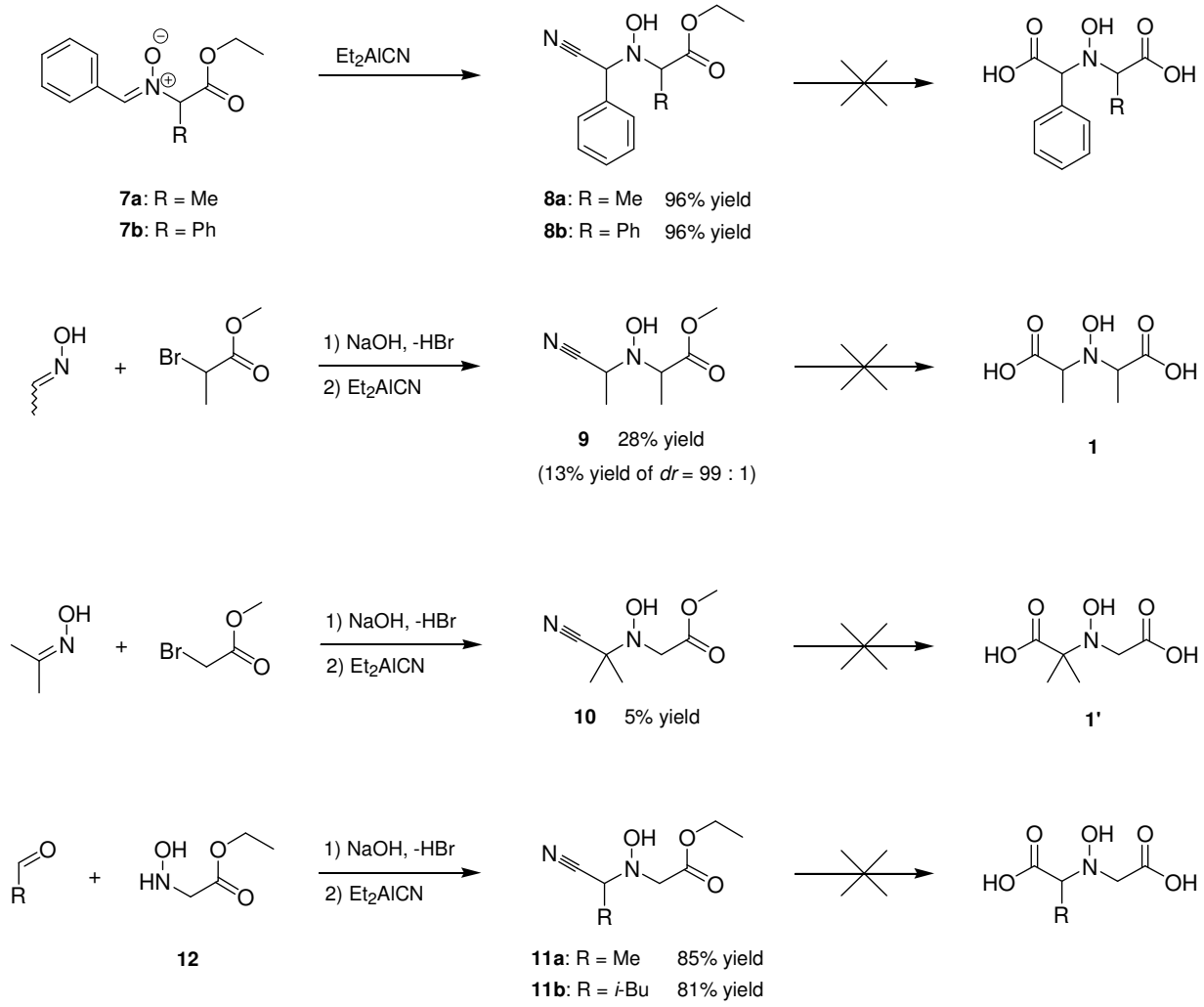
Scheme 3.8. *Retrosynthetic strategy based on two types of nitrone intermediates.*

3.3.2. Nitrone cyanation

The nitrones were prepared via either condensation or alkylation, and the cyanation reactions were performed according to the procedure reported by Merino *et al*.[45] The cyanating agent was diethylaluminum cyanide (Et_2AlCN), which has the potential to deliver the cyanide in a stereodivergent way and is commercially available as a 1 M solution in toluene. Moreover, it is highly reactive and its reactions with nitrones occur instantaneously.

Strategy (1): addition of cyanide to nitrones of type A. Two nitrones **7a** and **7b** were prepared via alkylation of *Z*-benzaldoxime according to the literature procedure.[54] Both were converted into the corresponding α-cyano secondary hydroxylamines **8a** and **8b** in 96% yield (Scheme 3.9). According to the integrals of the benzylic protons in ^{1}H NMR, both diastereomers of **8a** were present in almost equal amounts, while **8b** was formed in a *dr* of 3 : 1. Although the alkylation of oximes other than *Z*-benzaldoxime gives lower yields of the *N*-alkylated products, the method is still attractive because of the easy procedure and the use of commercially available starting materials. It was first applied for the reaction of acetaldoxime with methyl 2-bromopropionate, which are the building blocks to obtain the known amavadin ligand **1** via this route. An approximate 1 : 2 mixture of the nitrone and the oxime ether (NMR) was formed, from which isolation of the unstable nitrone proved difficult. However, the *in situ* reaction of the nitrone with Et_2AlCN yielded the cyanide adduct **9** in an overall yield of 28%. According to the integrals of the methyl protons in the NMR, both diastereomers of **9** were present in almost equal amounts. After fractional crystallisation, **9** was isolated as one

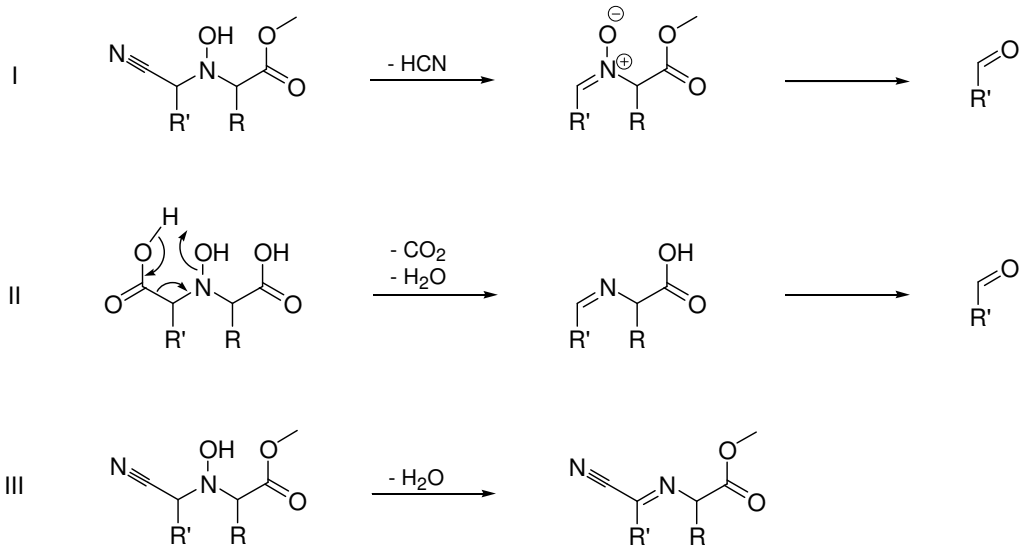
diastereomer in 13% yield with a *dr* of 99 : 1 (Scheme 3.9). Analogously, when acetone oxime was reacted with methyl bromoacetate, *in situ* nitrone cyanation gave the desired α-cyano hydroxylamine **10** in 5% yield as a crystalline material. Compound **10** is the required precursor for the production of the constitutional isomer **1'** of the amavadin ligand via this strategy.



Scheme 3.9. *Cyanide addition to isolated or in situ prepared nitrones.*

Among the many reports on nitrone synthesis via condensation, a few discuss the use of *N*-hydroxy amino acid esters.[55,56] Following these methods, two nitrones were prepared *in situ* from *N*-hydroxy glycine ethyl ester **12** and an aldehyde (i.e. acetaldehyde or isovaleraldehyde) in dichloromethane, using Na_2SO_4 as a drying agent. When TLC[57] showed that the condensation was complete, the Et_2AlCN solution was added to the reaction mixture (Scheme 3.9). The resulting α-cyano hydroxylamines **11a** and **11b** were isolated as crystalline compounds in good yields. Based on the promising results with these aldehydes, the reaction was also attempted with a ketone: acetone. However, the nitrone was not formed, even when acetone was used as the solvent or when other drying agents were used (molecular sieves, $MgSO_4$).

Having prepared the α-cyano secondary hydroxylamines **8**–**11** as described above, the final step in the synthesis remained: the hydrolysis of the nitrile and the ester groups. In the first instance, the hydrolysis was performed according to the common procedure for the hydrolysis of α-amino nitriles in the Strecker reaction: reflux in concentrated HCl.[58] This gave full conversion of the nitriles, but none of the desired ligands could be isolated from the resulting tars (Scheme 3.9). In the case of **8a** and **8b** benzaldehyde was identified, which indicates elimination of HCN from the starting material or decarboxylation of the product (routes I and II in Scheme 3.10, respectively). A third possibility of degradation is acid-induced elimination of water from the α-cyano hydroxylamine (route III).[47]



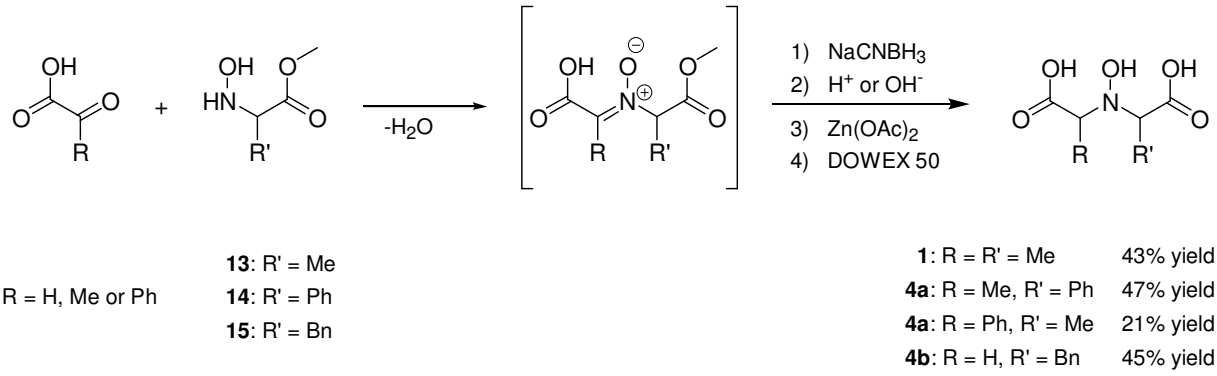
Scheme 3.10. *Possible degradation mechanisms during the attempted acid-catalysed nitrile hydrolysis.*

The application of milder conditions, according to the procedure used by Murahashi for α-cyano hydroxylamines[47] (4 M HCl with ice-cooling), did not give the desired products either. Recently, a platinum-based catalyst was described for the mild hydrolysis of nitriles to amides.[59] This catalyst was applied in the hydrolysis of the α-cyano secondary hydroxylamines, but none of the desired amides could be isolated. The starting compounds were not stable under the reaction conditions applied (80 °C in a water/ethanol mixture for several days), while **9** racemized before decomposition. The instability of the starting compounds seems to be the general reason for the failure of the hydrolyses. This is demonstrated by compound **9**, which would give the natural amavadin ligand **1** upon hydrolysis: the latter is stable under all conditions applied during the hydrolysis of **9**. The instability of **9** is also reflected in its racemisation under the relatively mild hydrolysis conditions, whereas **1** is conformationally stable, even in refluxing concentrated HCl.

3.3.3 Nitrone reduction

Our second approach to use nitrones as intermediates for the amavadin-based ligands comprised nitrone reduction. We prepared the nitrones by the condensation of an *N*-hydroxy amino acid ester with an aldehyde or ketone and *in situ* reduction using $NaCNBH_3$. This method may therefore be named a 'reductive hydroxylamination', stressing the analogy with the well-known reductive amination to obtain secondary amines.

Strategy (2): reduction of nitrones of type B. The required nitrones could be generated fast and effectively by using α-keto acids (Scheme 3.11). The choice for α-keto acids rather than the esters was based upon the faster condensation reaction that was observed for the acid with the *N*-hydroxy amino acid ester. By analogy to imine formation, this reaction is acid catalyzed. Furthermore, the ready availability of α-keto acids and their ease of handling are a merit.

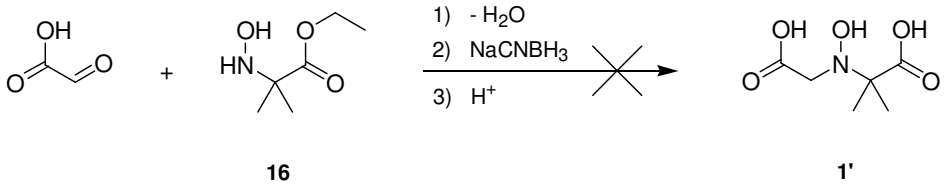


Scheme 3.11. *Synthesis of the amavadin ligand **1** and its analogues **4** via 'reductive hydroxylamination'.*

The first application of the method was the reaction of (racemic) *N*-hydroxy alanine methyl ester **13** with pyruvic acid in order to prepare the natural amavadin ligand **1**. Subsequent addition of $NaCNBH_3$ (instantaneous reaction) and evaporation of the solvent yielded a white solid, which was then refluxed in concentrated HCl to hydrolyze the ester. Further work-up was performed according to the procedure reported previously[2]: the crude ligand was precipitated with one equivalent of $Zn(OAc)_2$, followed by isolation of the intermediate zinc-salt (49% yield) and release of the pure ligand **1** via ion exchange chromatography and lyophilization. The overall yield of **1** was 43% and ^1H NMR showed a slight preference for the formation of the *meso*-isomer (*de* ≈ 20%). When starting from racemic *N*-hydroxy phenylglycine methyl ester **14** and pyruvic acid, the acid catalyzed ester hydrolysis completely degraded the product (smell of benzaldehyde), but when switching to alkaline conditions compound **4a** could be isolated in 47% yield as a mixture of two diastereomers in a ratio of 1 : 5. When the reaction sequence was performed with (racemic) *N*-hydroxy alanine methyl ester **13** and phenylglyoxylic acid, compound **4a** could be isolated as well. A benzyl group could be

introduced at the ligand backbone by starting with *N*-hydroxy phenylalanine methyl ester **15** and glyoxylic acid. After hydrolysis of the ester under reflux in 1 M HCl, the desired product **4b** was isolated in 41% yield. The use of optically pure **15** did not result in optically pure **4b**. Racemization of the chiral center is likely to take place in the nitrone intermediate, although the refluxing in hydrochloric acid could also induce such a racemization.

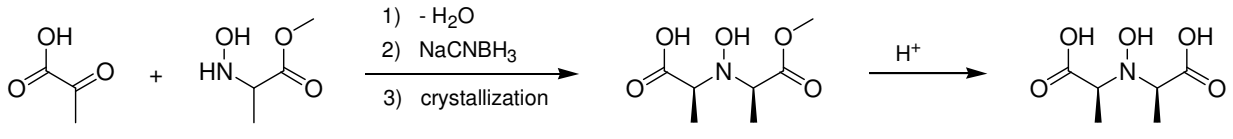
A third desired analogue was the constitutional isomer **1'** of the amavadin ligand, for which glyoxylic acid was reacted with α-methyl-*N*-hydroxy alanine ethyl ester **16** (Scheme 3.12). Unfortunately, the product did not precipitate with zinc and efforts to crystallize it from the reaction mixture failed. Instead, an impure oil was obtained in which only traces of the desired product could be detected (HR-MS).



16 **1'**

Scheme 3.12. *Attempted synthesis of the constitutional isomer **1'** of the amavadin ligand.*

An important feature of the method depicted in Scheme 3.11 is that the mono-ester **5** of the desired ligand is one of the intermediates. When performing the synthesis of ligand **1** via this method, we could isolate **5a** in a yield of 44% with a *dr* of 99 : 1 (NMR) by its crystallization from the quenched reaction mixture after the reduction step (Scheme 3.13). To identify its stereochemistry, **5a** was hydrolyzed to the amavadin ligand **1**. Comparison of the NMR data with those of enantiopure and racemic **1** revealed **5a** to be in the *meso*-form. Thus, next to *rac*-**5a** *meso*-**1** was prepared for the first time.

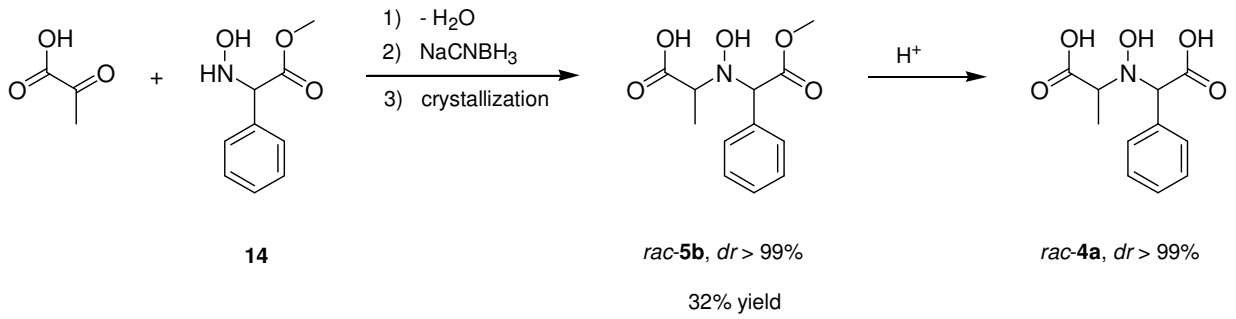


13 *rac*-**5a** *meso*-**1**

44% yield

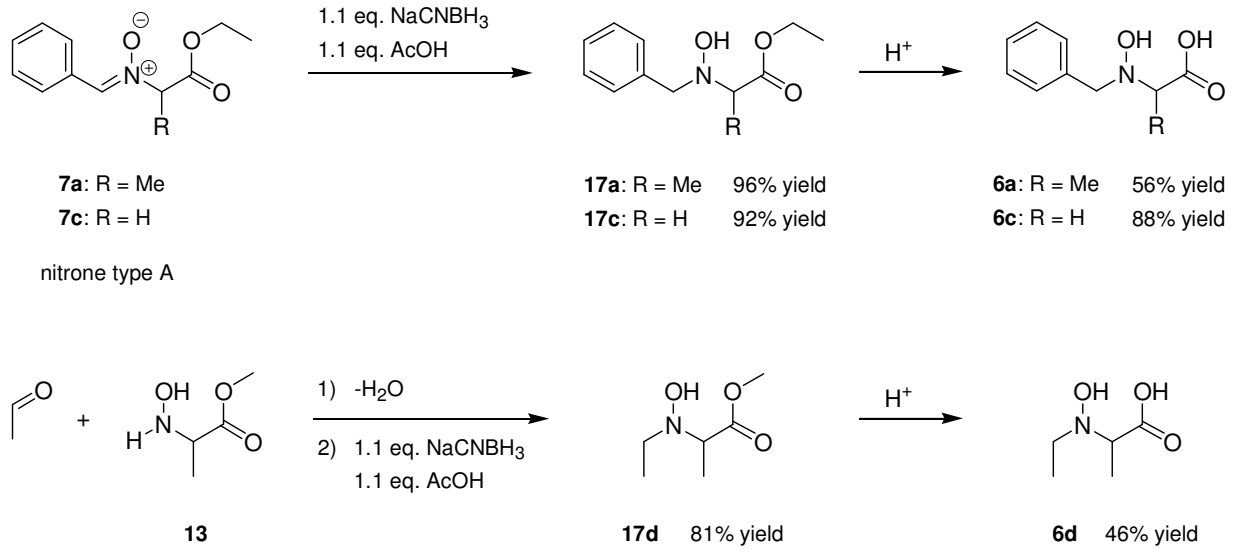
Scheme 3.13. *Synthesis of mono-ester **5a** and the identification of its stereochemistry.*

The reaction sequence was also performed for the combination of *N*-hydroxy phenylglycine methyl ester **14** and pyruvic acid, yielding mono-ester **5b** as one diastereomer (Scheme 3.14). Using 0.5 M NaOH solution, **5b** was hydrolyzed and the corresponding diacid **4a** was isolated as one diastereomer in 39% yield.



Scheme 3.14. *Synthesis of mono-ester **5b**.*

Strategy (3): reduction of nitrones of type A. The synthesis of *N*-alkylated *N*-hydroxy amino acids **6** could also be performed via the $NaCNBH_3$ reduction of the appropriate nitrones. The secondary hydroxylamines **17a** and **17c** thus obtained could be isolated in high yields. Subsequent hydrolysis gave the corresponding *N*-benzyl *N*-hydroxy amino acids **6a** and **6c** (Scheme 3.15). While the stable nitrones **7a** and **7c** could be isolated prior to their reduction, other nitrones can often only be prepared *in situ* and therefore require the 'reductive hydroxylamination' sequence. Accordingly, we generated the nitrone of acetaldehyde and **13**, followed by the *in situ* reduction to secondary hydroxylamine **17d**. Subsequent hydrolysis yielded *N*-ethyl *N*-hydroxy alanine **6d**.



Scheme 3.15. *Synthesis of N-alkylated N-hydroxy amino acids **6** using nitrone reduction.*

3.3.4. Evaluation of the application of nitrones as precursors for secondary hydroxylamines.

The synthesis of the α-cyano hydroxylamines is most successful if the intermediate nitrones can be prepared via the via *N*-alkylation of benzaldoxime (**7a** and **7b**) or via condensation of an aldehyde and an *N*-hydroxy amino acid ester (**10a** and **10b**). The *N*-alkylation of oximes other than benzaldoxime (**8** and **9**) has no general applicability because the *N/O*-alkylation ratios

with this method are generally too low. The synthesized α-cyano hydroxylamines **7**–**10** are the first to be based on a secondary *N*-hydroxy amino acid. Unfortunately, the hydrolysis of the nitriles to yield the corresponding amavadin ligand analogues remained a problem. If this obstacle is overcome, access to a large range of such analogues can be expected.

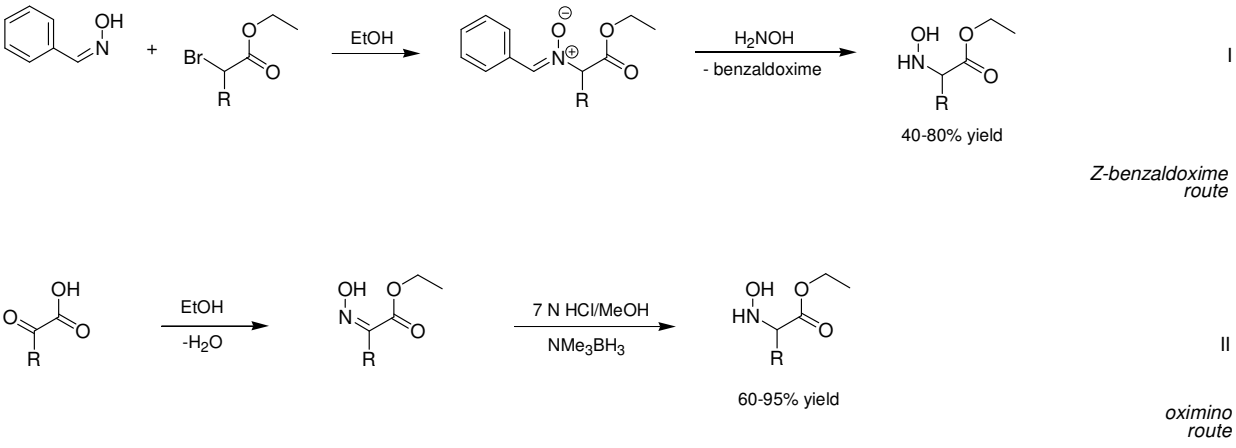
On the other hand, application of 'reductive hydroxylamination' to the synthesis of the amavadin-based ligands was successful. In a one-pot three-step procedure, we performed the non-stereoselective synthesis of the derivatives **4**, which are not accessible via the traditional method that is based on the *N*-alkylation of hydroxylamine. In addition, the strategy offered easy access to the mono-esters **5** as well as the *N*-alkylated-*N*-hydroxy amino acids **6**.

3.4. Synthesis of *N*-hydroxy amino acid esters.

The reductive hydroxylamination strongly depends on the availability of the appropriate hydroxylamine, which is a drawback of this method. The most recent review on the synthesis of *N*-hydroxy amino acid derivatives is from Ottenheijm, which dates from 1986.[19] A condensed overview of the available methods for the *N*-oxidation of amines appeared recently.[60] In this section, a brief overview of the available methods for the synthesis of *N*-hydroxy amino acid esters will be given (3.4.1), followed by our results on the application and the eventual modification of these routes (3.4.2). In the discussions, emphasis will be on their generality and ease of application.

3.4.1. Available methods for the synthesis of N-hydroxy amino acid esters

The review of Ottenheijm mentions the two traditional routes for the synthesis of *N*-hydroxy amino acid esters (Scheme 3.16). Both are of general applicability with yields of > 50%, which means that they outperform the other available routes.

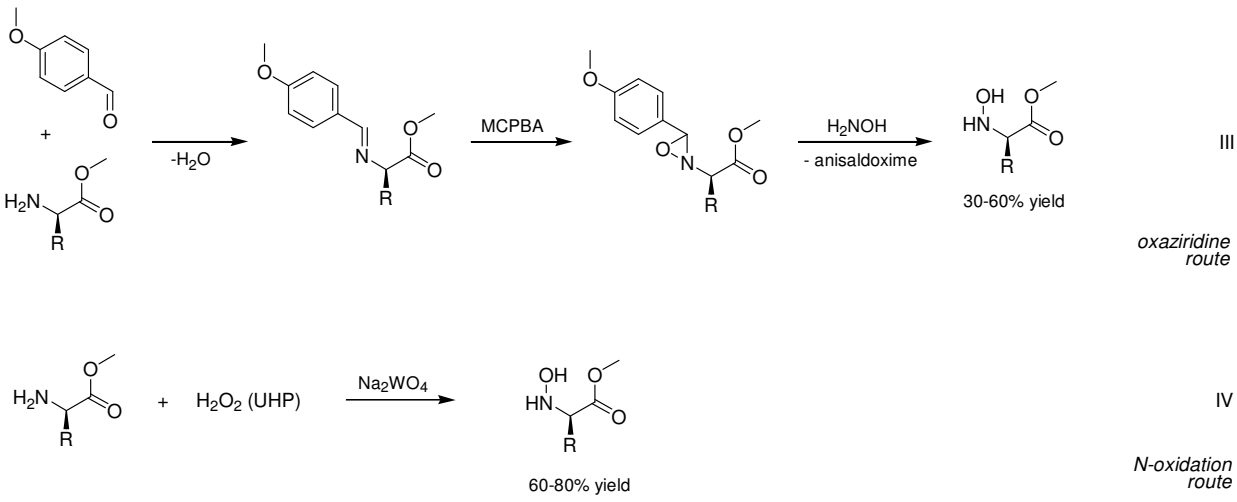


Scheme 3.16. *The two traditional routes for the synthesis of N-hydroxy amino acid esters.*

Route I is the earliest method and is based on the *N*-alkylation of *Z*-benzaldoxime,[61,62,54] followed by hydroxylaminolysis.[63] The use of *Z*-benzaldoxime is essential, because the *syn*-position of the phenyl group brings about alkylation on the hydroxylamine nitrogen, whereas *E*-benzaldoxime gives predominantly alkylation on the hydroxylamine oxygen. Route II was developed by the group of Ottenheijm and comprises the reduction of oximino compounds.[64,65]

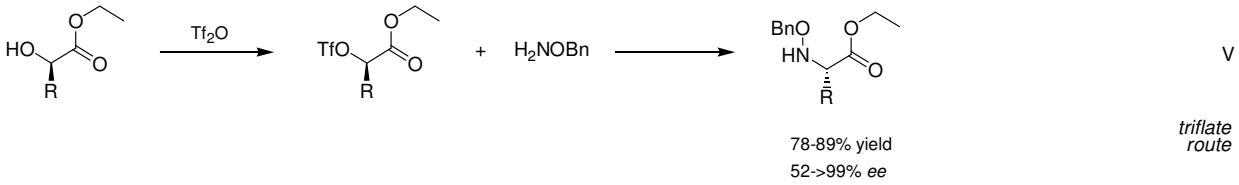
The drawback of route I is the use of the unstable *Z*-benzaldoxime, which easily reverts back to the low melting *E*-benzaldoxime during synthesis, work-up and handling. This problem was mainly overcome by Goto and coworkers[66] who used *Z*-2-furaldehyde oxime instead, which is thermodynamically more stable than the *E*-isomer. Surprisingly, their procedure did not receive much attention in later years, although its high yield, the general applicability, the possibilty of oxime-recycling and the easily accessible starting materials make it a very useful route. Furthermore, a unique feature of route I is that a broad range of higher *N*-hydroxy amino acid esters can be prepared by base-induced alkylation in the α-position of the N-O group of the isolated nitrone.[67] Route II has the advantage of easily accessible starting materials, although the harsh reaction conditions of the reduction (saturated HCl solution in methanol) make this route less attractive.

Another strategy to obtain *N*-hydroxy amino acid esters is the *N*-oxidation of the corresponding amine (Scheme 3.17). In 1974, an indirect *N*-oxidation route was reported,[68,69] involving imine formation, oxidation, and hydroxylaminolysis of the oxaziridine (route III). Very recently, a direct *N*-oxidation procedure of primary amines with the urea-hydrogen peroxide complex (UHP) was reported[60] using sodium tungstate as a catalyst in the absence of water (route IV).



Scheme 3.17. *Synthesis of chiral N-hydroxy amino acid esters via indirect (III) and direct (IV) N-oxidation.*

Whereas routes I and II produce racemic mixtures, routes III and IV can be used for the preparation of optically pure *N*-hydroxy amino acid esters, as the chiral center is not affected during the oxidation and hydrolysis steps. Moreover, these routes have a much broader scope as they can be applied in the synthesis of chiral primary *N*-hydroxy compounds in general. A third asymmetric synthesis method is the stereoselective reaction of *O*-benzyl protected hydroxylamine with chiral α-triflate-esters, developed by the group of Ottenheijm[22] (route V, Scheme 3.18). Excellent *ee*'s (>99%) and high chemical yields (up to 89%) were obtained for the *N*-hydroxy amino acid esters with R = Me, Bn and *i*-Pr. In the case of R = Ph the *ee* decreased to 52%.

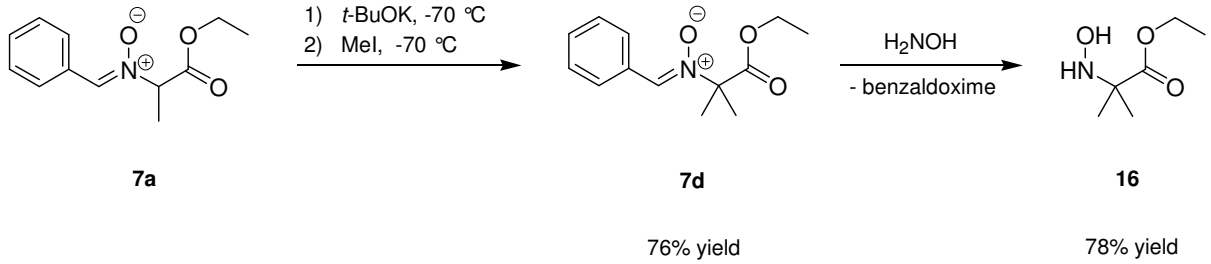


Scheme 3.18. *Synthesis of chiral N-hydroxy amino acid esters via substitution on triflates.*

3.4.2. Synthesis of N-hydroxy amino acid esters.

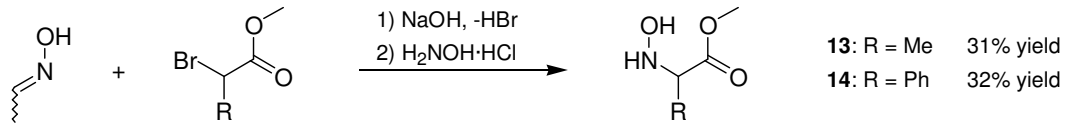
For the preparation of the required *N*-hydroxy amino acid esters, we applied all routes mentioned above. It was not our primary aim to try and compare these routes, but the red line in this project set the scene to give these routes a try for a full comparison. Namely, we synthesized the intermediate nitrones of route I for the cyanide additions (Scheme 3.9), while the oximino compounds of route II were prepared in order to investigate if *N*-alkylation is a feasible route to obtain nitrones of type B (Scheme 3.11). Furthermore, route V is part of the enantioselective synthesis of the diester of the amavadin ligand (Chapter 2).

Route I: hydroxylaminolysis of *N*-alkylated *Z*-benzaldoxime. The large scale synthesis of *Z*-benzaldoxime was performed with substantially lower yields than the literature procedure, 12% *vs* 35%. Suppression of the reversion to the *E*-form during work-up and handling appeared to be a problem, judging from the large amounts of *E*-benzaldoxime that were formed. In the next step, the nitrones could easily be synthesized and converted into the *N*-hydroxy amino acid esters by hydroxylaminolysis, in yields comparable to those reported in the literature. Following the report of Schöllkopf and Lau on the methyl esters,[67] nitrone **7d** could be prepared via α-alkylation of nitrone **7a** (Scheme 3.19), from which the α-methyl-*N*-hydroxy alanine ethyl ester **16** was obtained in good yield.



Scheme 3.19. *Synthesis of **16** via base-induced alkylation on the α-position of nitrone **7a**.*

Furthermore, a modification of route I was envisioned during the investigation of oxime alkylations. It was shown in section 3.3.2 that reaction of acetaldoxime with methyl 2-bromopropionate yields an approximate 1 : 2 mixture of the nitrone and the oxime ether. This is a moderate selectivity towards *N*-alkylation, but this yield is higher than the overall yield in the two-step reaction starting from *E*-benzaldoxime. Therefore, instead of an *in situ* cyanation, an *in situ* hydroxylaminolysis was performed on the nitrone, after which the corresponding *N*-hydroxy amino acid esters **13** and **14** could be isolated in high purity (Scheme 3.20). This three-step one-pot procedure starts from cheap and commercially available compounds, making it a convenient and effective method.



Scheme 3.20. *A new three-step one-pot procedure for the synthesis of N-hydroxy amino acid esters.*

Route II: reduction of oximino compounds. This route was only applied for the synthesis of *N*-hydroxy alanine methyl ester, but the use of 7 M HCl in MeOH was rather inconvenient. The preparation of this mixture is unpleasant, as well as the work-up (evaporation of the solvent). With this method, a yield (65%) comparable with the literature yield (71%) was obtained.

Route III: hydroxylaminolysis of oxaziridines. This is a more laborious method, and yields were moderate, as in the literature. However, the chiral starting compounds are cheap, the application on large scale is no problem and the products are indeed optically pure. With this method, (*R*)-*N*-hydroxy phenylalanine methyl ester **15** was prepared (27% yield), which was the chiral starting material in the synthesis of the amavadin ligand analogue **4b**.

Route IV: direct *N*-oxidation of amines. This method could not be reproduced, unfortunately. The success that the authors claim in their publication is remarkable because the direct oxidation of primary amines that stops at the hydroxylamine stage is generally considered very

difficult. The different catalytic activity of the heterogeneous Na_2WO_4 might be due to different particle size or other material parameters not provided.

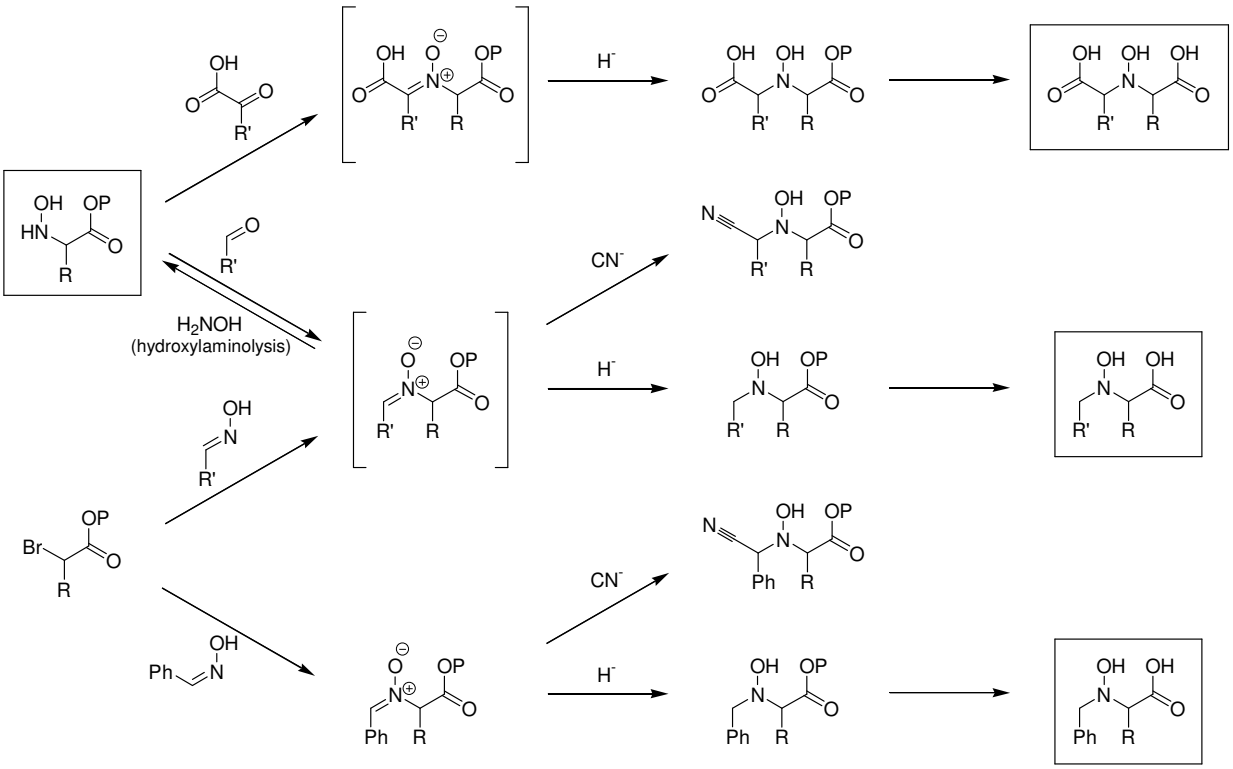
Route V: alkylation of hydroxylamines using a triflate leaving group. This route was used to prepare optically pure *N*-hydroxy alanine ethyl ester. The compound was isolated in high yield in excellent *ee*.

3.4.3. Comparison

We applied the available synthetic methods for *N*-hydroxy amino acid esters, and it appeared that the synthesis of most of the esters required an individual approach. The only method of general applicability would be the direct *N*-oxidation of amino acids, were it not for the fact that its reproduction is difficult. It can be stated that in its basics, the most versatile route is the *N*-alkylation route, as it allows the preparation of higher *N*-hydroxy amino acid derivatives by the base-induced alkylation on the α-position of the nitrone. For the synthesis of chiral *N*-hydroxy amino acid esters, the indirect *N*-oxidation seems the best strategy.

3.5. Conclusions

We showed that a range of secondary hydroxylamines is accessible via either cyanation or reduction of nitrones that are based on *N*-hydroxy amino acid esters (Scheme 3.21).



Scheme 3.21. *Overview of the primary and secondary hydroxylamines obtained via nitrones.*

The most important result is that a synthesis route towards the amavadin-based ligands **4**, **5** and **6** has been developed, that has broader applicability than the traditional methods. Secondly, the synthesis of six new α-cyano sedondary hydroxylamines has been performed. Unfortunately, it was not possible to convert these into the corresponding amavadin-based ligands. An interesting spin-off of the work on nitrones is the effective and easily applicable method to obtain racemic *N*-hydroxy amino acid esters.

3.6. Experimental

General considerations. Column chromatography was carried out with silica gel 0.060-0.200 mm, pore diameter approx. 6 nm (Fluka silica gel 60). TLC was performed on 0.20 mm silica gel aluminium sheets (Merck silica gel 60 F_{254}). Elution was carried out with mixtures of petrolether (PE; boiling point range of 40–65 °C) and ethyl acetate (EtOAc). The plates were developed by dipping either in a ninhydrin bath (1.5 g of ninhydrin in 100 mL of ethanol) where all hydroxylamines gave pale red to yellow spots or in a iodine/silica gel bath (prepared as a mixture of 5 g of iodine and 300 g of silica gel). Ion exchange chromatography was carried out with DOWEX 50WX8-200. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-400S (400 MHz) or a Varian Unity Inova 300 (300 MHz) instrument. Chemical shifts (δ) are expressed in parts per million relative to tetramethylsilane (in $CDCl_3$ or DMSO) or *t*-BuOH (in H_2O). Coupling constants (*J*) are expressed in Hertz. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet). Low resolution mass spectroscopy was measured on a Micromass Quattro LC-MS spectrometer (Electron Spray Ionization). High resolution mass spectroscopy was measured on a Thermo LTQ Orbitrap; resolution 50000; mass accuracy <2 ppm; samples were diluted with a 1 : 1 methanol/water solution; injection volume: 10 µl; flowrate: 50 µl/min; eluent is a 1 : 1 methanol/water solution. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Melting points were measured on a Büchi 510 and are uncorrected. The C, H and N elemental analyses were performed at the Dr. Verwey Chemical Laboratory in Rotterdam, The Netherlands. The α-bromoacids[70,71] and the α-hydroxy acid esters[72,73] were prepared according to standard literature procedures. All other chemicals and dry solvents were obtained from Aldrich or Merck and were used as received. Other solvents were purchased from Baker. Ultrapure water (MilliQ) was used.

Synthesis of amavadin ligands 1 and 2 via substitution on α-bromo acids. The synthesis was performed according to the literature procedure with modifications.[29] A pH-controlled automatic burette was charged with a 4 M NaOH solution. With the aid of this set-up, a stirred solution of hydroxylamine hydrochloride and the α-bromo acid in water was brought to pH 7.2. At this point, the reaction started and the pH of the reaction mixture was kept at 7.2 by the controlled addition of a 4 M NaOH solution, while the temperature was kept at 30–35 °C. When the reaction was complete, the pH of the mixture was brought to 4.4 with diluted HCl. A solution of the $Zn(OAc)_2{\cdot}2H_2O$ in 40 mL of water was added to precipitate the ligand. The milk-white suspension was washed with water (a cycle of

centrifugation, decanting the supernatant and suspending the residue in water was performed three times) and dried *in vacuo*, after which the zinc complex was obtained as a fine white powder. It was then dissolved in 20 mL of 6 M HCl (or the minimum amount that is necessary for complete dissolution) and the solution was brought onto a column of H^+-loaded DOWEX-50 ion exchange resin that was previously washed with water until neutral pH. Elution with water first gave an eluate with a pH of <1, then the pH increased to 3.4, and when the pH was decreasing again the eluate was collected. After concentration and lyophilization a white powder was obtained.

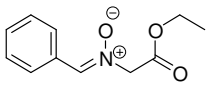
1. From 19.122 g (125 mmol) of (*R*)-2-bromopropionate, 3.474 g (50 mmol) of hydroxylamine hydrochloride and 10.975 g (50 mmol) of $Zn(OAc)_2{\cdot}2H_2O$, 6.841 g (28.44 mmol, 57%) of the zinc complex was obtained. The reaction was carried out in 110 mL of water and was complete after 40 hours. Ion exchange chromatography yielded 4.312 g (24.34 mmol, 49% overall yield) of **1**. Mp = 137–138 °C. $[\alpha]_D^{20}$ = +21.3° (c=1, H_2O). MS (*m*/*z*) 178.3 (ES^+, M+1), 176.3 (ES^-, M–1). NMR showed that the ratio of *meso*-**1** : **1** was approximately 1 : 4 (*de* = 60%). The ^1H and ^{13}C NMR of **1** were identical to those of **1** described in the literature.[2] The ^1H and ^{13}C NMR *meso*-**1** were identical to those of *meso*-**1** described below.

2. From 34.738 g (250 mmol) of bromoacetic acid, 6.949 g (100 mmol) of hydroxylamine hydrochloride and 21.950 g (100 mmol) of $Zn(OAc)_2{\cdot}2H_2O$, 11.600 g (54.6 mmol, 55%) of the zinc complex was obtained. The reaction was carried out in 150 mL of water at RT instead of 30–35 °C and was complete after 24 hours. The release of **2** was performed batchwise; from 2.125 g (10 mmol) of the zinc complex, 1.180 g (7.91 mmol, 79%) of **2** was obtained as a white solid (43% starting from bromoacetic acid and hydroxylamine hydrochloride). Mp = 137 °C. ^1H NMR (D_2O, 300 MHz, 25 °C) δ; 3.80 (s, 4H, 2 x NC<u>H</u>$_2$), ^{13}C NMR (D_2O, 75 MHz, 25 °C) δ; 173.33 (2C, 2 x <u>C</u>OOH), 61.49 (2C, 2 x N<u>C</u>H_2). MS (*m*/*z*) 150.3 (ES^+, M+1), 148.3 (ES^-, M–1). Anal. calcd for $C_4H_7NO_5$: C, 32.22; H, 4.73; N, 9.39. Found: C, 32.30; H, 4.59; N, 9.28.

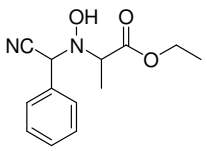
Synthesis of nitrones 7. The nitrones **7a**, **7b** and **7c** were synthesized and isolated according to the literature procedures.[54,74]

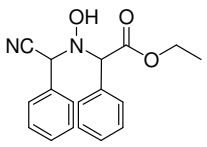
7a. Yield: 48%, white crystals. Mp = 112 °C. ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.28 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 1.78 (d, 3H, $CHC\underline{H}_3$, *J* = 7.0), 4.26 (m, 2H, $C\underline{H}_2CH_3$, *J* = 7.1), 4.75 (q, 1H, $C\underline{H}CH_3$, *J* = 7.0), 7.42 (m, 3H, $C_6\underline{H}_5$), 7.48 (s, 1H, N=CH), 8.25 (m, 2H, $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 167.96 (1C, <u>C</u>OOEt), 134.95 (1C, N=<u>C</u>H), 130.72, 130.27, 128.86, 128.54 (6C, <u>C</u>$_6H_5$), 73.30 (1C, <u>C</u>HCH_3), 62.26 (1C, O<u>C</u>H_2CH_3), 15.61 (1C, $OCH_2\underline{C}H_3$), 14.03 (1C, $CH\underline{C}H_3$).

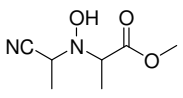
7b. Yield: 42%, white crystals. Mp = 114 °C. ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.30 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 4.33 (m, 2H, $C\underline{H}_2CH_3$, *J* = 7.1), 5.82 (s, 1H, $C\underline{H}C_6H_5$), 7.39 (m, 8H, 2 x $C_6\underline{H}_5$), 7.49 (s, 1H, N=CH), 8.17 (m, 2H, $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 167.10 (1C, <u>C</u>OOEt), 135.76 (1C, N=<u>C</u>H), 131.12, 130.98, 130.25, 129.85, 129.71, 129.43, 129.18, 128.44 (12C, <u>C</u>$_6H_5$), 81.71 (1C, <u>C</u>HC_6H_5), 62.53 (1C, O<u>C</u>H_2CH_3), 14.03 (1C, $OCH_2\underline{C}H_3$).

7c. Yield: 60%, white crystals. Mp = 30 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.31 (t, 3H, 
$CH_2C\underline{H}_3$, *J* = 7.2), 4.28 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.2), 4.71 (s, 2H, $NC\underline{H}_2$), 7.42 (m, 3H, $C_6\underline{H}_5$), 7.43 (s, 1H, N=CH), 8.24 (m, 2H, $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 165.70 (1C, $\underline{C}$OOEt), 137.13 (1C, N=$\underline{C}$H), 130.96, 130.08, 128.84, 128.54 (6C, $\underline{C}_6H_5$), 68.14 (1C, $N\underline{C}H_2$), 62.34 (1C, $O\underline{C}H_2CH_3$), 14.07 (1C, $OCH_2\underline{C}H_3$).

Synthesis of α-cyano secondary hydroxylamines 8a and 8b. To a solution of the nitrone in 40 mL of dry CH_2Cl_2 under a nitrogen atmosphere was added the 1 M Et_2AlCN solution. The mixture was stirred for 10 minutes after which TLC (PE/EtOAc 8:2) showed full conversion of the nitrone. After the addition of 50 mL of saturated $NaHCO_3$ and 10 min of fast stirring (viscous suspension), the water layer was extracted with 3x30 mL of CH_2Cl_2. The collected extracts were dried over Na_2SO_4 and traces of toluene needed to be removed by stripping with dichloromethane.

8a. From 1.921 g (8.68 mmol) of **7a** and 9.12 mL (9.12 mmol) of 1M $AlEt_2CN$ toluene solution, 2.069 
g (8.33 mmol, 96%) of **8a** was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.29, 1.30 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 1.45, 1.47 (d, 3H, $CHC\underline{H}_3$, *J* = 7.0), 3.73, 3.74 (q, 1H, $C\underline{H}CH_3$, *J* = 7.1), 4.20, 4.22 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.0), 5.12, 5.13 (s, 1H, $C\underline{H}C_6H_5$), 5.63, 5.68 (s, 1H, NO$\underline{H}$), 7.42 (m, 3H, $C_6\underline{H}_5$), 7.56 (m, 2H, $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 172.04, 171.91 (1C, $\underline{C}$OOEt), 132.54, 132.31, 129.47, 129.38, 128.97, 128.91, 128.77, 128.69, (6C, $\underline{C}_6H_5$), 116.40, 115.73 (1C, N≡$\underline{C}$), 62.96, 62.29, 61.40, 61.29, 61.19, 59.84 (3C, $\underline{C}HCH_3$ + $O\underline{C}H_2CH_3$ + $\underline{C}HC_6H_5$), 15.25, 14.73 (1C, $CH\underline{C}H_3$ + $OCH_2\underline{C}H_3$), 14.12 (1C, $CH\underline{C}H_3$ + $OCH_2\underline{C}H_3$). Anal. calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.79; H, 6.32; N, 11.38.

8b. From 629 mg (2.22 mmol) of **7b** and 2.33 mL (2.33 mmol) of 1M $AlEt_2CN$ toluene solution, 659 
mg (2.12 mmol, 96%) of **8b** was obtained as white crystals. Mp = 142–143 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.17 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 4.10 (m, 2H, $C\underline{H}_2CH_3$), 4.59, 4.80 (2 x s, 2H, $C\underline{H}C_6H_5$), 5.81 (s, 1H, NO$\underline{H}$), 7.36–7.47 (m, 8H, 2 x $C_6\underline{H}_5$), 7.59–7.62 (m, 2H, 2 x $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 170.09 (1C, $\underline{C}$OOEt), 133.57, 132.80, 130.01, 129.72, 129.22, 128.84, 128.53 (12C, 2 x $\underline{C}_6H_5$), 115.05 (1C, N≡$\underline{C}$), 74.30, (1C, N≡$C\underline{C}$H), 61.78, 59.41 (2C, $O\underline{C}H_2CH_3$ + $\underline{C}HC_6H_5$), 14.13 (1C, $OCH_2\underline{C}H_3$). Anal. calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.57; H, 5.71; N, 8.81.

Synthesis of α-cyano secondary hydroxylamine 9. Under a nitrogen atmosphere, 0.644 g (28.00 
mmol) of sodium was dissolved in 50 mL of dry methanol. After the addition of 1.654 g (28.00 mmol) of acetaldoxime and 4.911 g (29.41 mmol) of methyl 2-bromopropionate, the mixture was stirred overnight. By then, the pH of the solution had decreased to almost neutral, as indicated by wet pH-paper. Because of the instability of the nitrone, subsequent steps were performed quickly. The methanol was evaporated and CH_2Cl_2 was added to precipitate the NaBr, which was then removed by filtration. Evaporation yielded a mixture of the nitrone and the oxime ether as a yellow oil. Petrolether was added to form a bi-phasic system and the oxime ether was separated from the nitrone by removing the colourless upperlayer using a pipet. The

remaining yellow oil was dissolved in 50 mL of CH_2Cl_2 under a nitrogen atmosphere and 8 mL (8 mmol) of 1M $AlEt_2CN$ toluene solution was added in one portion. TLC (PE/EtOAc 8:2) showed a complete and immediate conversion of the nitrone (Rf <0.05; strong UV absorbtion) and formation of a new product (Rf = 0.4; ninhydrin). After the addition of 50 mL of saturated $NaHCO_3$ and 10 min of fast stirring (viscous suspension), the water layer was extracted with 3x30 mL of CH_2Cl_2. The collected extracts were dried over Na_2SO_4 and traces of toluene and the oxime ether were removed by stripping with CH_2Cl_2. The resulting yellow oil became a solid at –18 °C, and after washing with a mixture of petrolether/diethyl ether 1.363 g (7.92 mmol, 28%) of **9** was obtained as white crystals. Recrystallization gave 0.605 g (3.51 mmol, 13%) of **9** as a single diastereoisomer. Mp = 107–108 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,) δ; 1.35 (d, 3H, NCHC<u>H</u>$_3$, *J* = 7.0), 1.61 (d, 3H, NCHC<u>H</u>$_3$, *J* = 6.9), 3.76 (q, 1H, NC<u>H</u>CH$_3$, *J* = 6.9), 3.77 (s, 3H, OC<u>H</u>$_3$), 3.94 (q, 1H, NC<u>H</u>CH$_3$, *J* = 7.0), 6.23 (s, 1H, NO<u>H</u>). ^{13}C NMR ($CDCl_3$, 75 MHz) δ; 172.84 (C, <u>C</u>OOEt), 116.85 (1C, N≡<u>C</u>), 64.34 (1C, N<u>C</u>HCH$_3$), 52.36 (1C, O<u>C</u>H$_3$), 49.99 (1C, N<u>C</u>HCH$_3$), 17.12, 15.03 (2C, NCH<u>C</u>H$_3$). Anal. calcd for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.02; N, 16.27. Found: C, 48.87; H, 6.99; N, 16.18.

Synthesis of α-cyano secondary hydroxylamine 10. The same procedure as for the synthesis of **10** was followed. By reacting 0.841 g (36.60 mmol) of sodium, 5.821 g (38.05 mmol) of methyl bromoacetate and 2.649 g (36.24 mmol) acetone oxime, the formation of the intermediate nitrone was complete within 2 hours. After its isolation as a yellow oil and the immediate addition of 4.5 mL (4.5 mmol) of the 1M $AlEt_2CN$ toluene solution, 319 mg (1.85 mmol, 5%) of **10** was isolated as white crystals. Mp = 95 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.56 (s, 6H, NC(C<u>H</u>$_3$)$_2$), 3.68 (s, 2H, NC<u>H</u>$_2$), 3.78 (s, 3H, OC<u>H</u>$_3$), 6.48 (s, 1H, NO<u>H</u>). ^{13}C NMR ($CDCl_3$, 75 MHz) δ; 170.34 (C, <u>C</u>OOMe), 119.90 (1C, N≡<u>C</u>), 59.53, 56.19 (2C, N<u>C</u>(CH$_3$)$_2$, N<u>C</u>H$_2$), 52.51 (1C, O<u>C</u>H$_3$), 25.41 (2C, N(<u>C</u>H$_3$)$_2$). Anal. calcd for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.02; N, 16.27. Found: C, 48.91; H, 6.99; N, 16.12.

Synthesis of α-cyano secondary hydroxylamines 11a and 11b. Under a nitrogen atmosphere, the *N*-hydroxy glycine ethyl ester and the aldehyde were condensed in 20 mL of CH_2Cl_2 with the aid of Na_2SO_4 drying agent. When TLC (PE/EtOAc 1:1) showed full conversion of the ester to the intermediate nitrone (Rf <0.05; strong UV absorbtion), the 1M $AlEt_2CN$ toluene solution was added in one portion. TLC showed a complete and immediate conversion of the nitrone and formation of a new product. After the addition of 50 mL of saturated $NaHCO_3$ and 10 min of fast stirring (viscous suspension), the water layer was extracted with 3x30 mL of CH_2Cl_2. The collected extracts were dried over Na_2SO_4 and traces of toluene were removed by stripping with petrolether. The resulting yellow oil became a solid at –18 °C, which was purified by recrystallization from a mixture of petrolether/diethyl ether.

11a. From 0.350 g (2.94 mmol) of *N*-hydroxy glycine ethyl ester, 0.142 g (3.23 mmol) of acetaldehyde and 3.4 mL (3.4 mmol) of 1M $AlEt_2CN$ toluene solution, 430 mg (2.50 mmol, 85%) of **11a** was obtained as white crystals. During the condensation, the mixture was cooled with ice to prevent evaporation of the acetaldehyde. Initially, the

condensation reaction proceeded slowly, but it could be promoted by the addition of one drop of diluted HCl and was complete after four hours. Mp = 35–36 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.30 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.2), 1.56 (d, 3H, $CHC\underline{H}_3$, *J* = 7.2), 3.55 (1H, $NC\underline{H}_2$, *J* = 16.5), 3.75 (1H, $NC\underline{H}_2$, *J* = 16.5), 3.90 (q, 1H, $C\underline{H}CH_3$, *J* = 7.2), 4.23 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.2), 6.73 (s, 1H, NO<u>H</u>), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 169.48 (1C, <u>C</u>OOEt), 117.39 (1C, N≡<u>C</u>), 61.69 (1C, O<u>C</u>H_2CH_3), 58.70, 54.30 (2C, N<u>C</u>H_2 + N<u>C</u>HCN), 17.02 (1C, CH<u>C</u>H_3), 14.34 (1C, OCH$_2$<u>C</u>H_3). Anal. calcd for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.02; N, 16.27. Found: C, 48.94; H, 6.99; N, 16.02.

11b. From 0.119 g (1.00 mmol) of *N*-hydroxy glycine ethyl ester, 0.095 g (1.10 mmol) of freshly distilled valeraldehyde and 1.15 mL (1.15 mmol) of 1M $AlEt_2CN$ toluene solution, 173 mg (0.81 mmol, 81%) of **11b** was obtained as white crystals. The condensation was complete after one hour. Mp = 87 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 0.96, 0.99 (2 x d, 6H, CH(C<u>H</u>$_3$)$_2$), 1.30 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.2), 1.69–1.97 (m, 3H, NCC<u>H</u>$_2$ + C<u>H</u>(CH$_3$)$_2$), 3.55 (1H, $NC\underline{H}_2$, *J* = 16.5), 3.76 (1H, $NC\underline{H}_2$, *J* = 16.5), 3.82 (dd, 1H, NC<u>H</u>), 4.23 (q, 2H, OC<u>H</u>$_2$, *J* = 7.2), 6.40 (s, 1H, NO<u>H</u>), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 169.27 (1C, <u>C</u>OOEt), 116.70 (1C, N≡<u>C</u>), 61.40 (1C, O<u>C</u>H_2CH_3), 58.60, 57.76 (2C, N<u>C</u>H_2 + N<u>C</u>HCN), 39.48, 24.84, 22.52, 21.91 (4C, <u>C</u>H$_2$<u>C</u>H(<u>C</u>H$_3$)$_2$), 14.13 (1C, OCH$_2$<u>C</u>H_3). Anal. calcd for $C_{10}H_{18}N_2O_3$: C, 56.06; H, 8.47; N, 13.07. Found: C, 56.04; H, 8.44; N, 12.95.

Synthesis of 1, 1', 4a and 4b via reductive hydroxylamination: The *N*-hydroxy amino acid ester and the α-keto acid were stirred in methanol until TLC (PE/EtOAc 1:1) showed full conversion of the *N*-hydroxy amino acid ester. After the addition of $NaCNBH_3$, TLC showed a complete and immediate conversion of the nitrone (Rf <0.05; strong UV absorbtion). Evaporation of the methanol yielded a white solid, which was then hydrolysed under acidic (**1**, and **4b**) or basic (**4a**) conditions. The $Zn(OAc)_2{\cdot}2H_2O$ was added to precipitate the ligand and the resulting milk-white suspension was washed with water (a cycle of centrifugation, decanting the supernatant and suspending the residue in water was performed three times) and dried *in vacuo*. It was then dissolved in 3 M HCl (using the minimum amount that is necessary for complete dissolution) and it was brought onto a column of H^+-loaded DOWEX-50 ion exchange resin that was previously washed with water until neutral pH. Elution with water first gave an eluate with a pH of <1, after which the pH increased again. When the pH started to decrease for the second time, the eluate was collected. After concentration, the solution was either lyophilized (**1**) or evaporated to dryness and stripped with CH_2Cl_2 (**4a** and **4b**; both are slightly soluble in CH_2Cl_2).

1. The synthesis was performed using 357 mg (3.00 mmol) of **13**, 280 mg (3.18 mmol) of pyruvic acid, 211 mg (3.36 mmol) of $NaCNBH_3$ and 659 mg (3.00 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. The conditions applied for the hydrolysis were stirring for 6 hours in 11 mL 10 M HCl at 110 °C. After evaporation of the aqueous HCl, the mixture was dissolved in water and the pH was adjusted to 4.4 with aqueous NH_4OH before adding the $Zn(OAc)_2{\cdot}2H_2O$. The zinc complex was obtained as a fine white powder in a yield of 350 mg (1.46 mmol, 49%), from which **1** was obtained in an overall yield of 229 mg (1.29 mmol, 43%). Mp = 120 °C (decomp.). ^{1}H and ^{13}C NMR showed the presence of the two diastereomers in a ratio of A : B = 6 : 4, in which A is the

meso-compound. In all other respects, ^{1}H NMR, ^{13}C NMR, and MS were identical to those of **1** obtained via the substitution on 2-bromopropionate as described above.

Attempted synthesis of 1'. The synthesis was performed using 365 mg (2.48 mmol) of *N*-hydroxy α-methyl alanine ethyl ester, 242 mg (2.63 mmol) of glyoxylic acid·H_2O, 175 mg (2.78 mmol) of $NaCNBH_3$ and 544 mg (2.48 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. During the condensation step the use of $MgSO_4$ as a drying agent appeared necessary. The conditions applied for the hydrolysis were stirring for 1 hour in 15 mL 3 M HCl at 125 °C. After evaporation of the aqueous HCl, the mixture was dissolved in water and the pH was then adjusted to 1.80 with aqueous NH_4OH after which the mixture was concentrated. Attempts to crystallize **1'** from the raw reaction mixture failed, neither did a precipitate form upon the addition of $Zn(OAc)_2{\cdot}2H_2O$.

4a (starting from 14). The synthesis was performed using 336 mg (1.85 mmol) of **14**, 171 mg (1.94 mmol) of pyruvic acid, 128 mg (2.04 mmol) of $NaCNBH_3$ and 407 mg (1.85 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. The conditions applied for the hydrolysis were stirring for 5 hours in 0.5 M NaOH at RT. The pH was then adjusted to 1.80 with aqueous HCl after which the mixture was concentrated. Precipitation with $Zn(OAc)_2{\cdot}2H_2O$ yielded 265 mg (0.88 mmol, 47%) of the zinc complex as a fine white powder, from which **4a** was obtained as a white solid in an overall yield of 172 mg (0.72 mmol, 47%). NMR showed the presence of **4a** as two isomers in a ratio of A : B = 1 : 5. The assignment of the ^{1}H and ^{13}C resonances was based on this ratio. Mp = 115 °C (decomp.). ^{1}H NMR (DMSO, 300 MHz, 25 °C) δ; 1.13^{B}, 1.35^{A} (d, 3H, CHC$\underline{H}_3$, J = 6.9), 3.09^{B}, 3.24^{A} (q, 1H, C$\underline{H}$CH$_3$, J = 6.9), 4.54^{A}, 4.99^{B} (s, 1H, C$\underline{H}$C$_6$H$_5$), $7.33–7.50^{A+B}$ (m, 5H, C$_6\underline{H}_5$). ^{13}C NMR (DMSO, 75 MHz, 25 °C): δ 173.12^{B}, 172.83^{B} (2C, 2 x $\underline{C}$OOH), 136.84^{B}, 129.47^{B}, 129.21^{B}, 129.09^{B} (6C, $\underline{C}_6H_5$), 73.23^{B} (1C, $\underline{C}$HC$_6$H$_5$), 59.11^{B} (1C, $\underline{C}$HCH$_3$), 16.66^{B} (1C, CH$\underline{C}$H$_3$). HRMS-ESI calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$ 240.0867, found 240.0863.

4a (starting from 13). The procedure was identical to the one used for the synthesis of **4a** starting from **14**, but during the condensation step the use of $MgSO_4$ as a drying agent appeared necessary. The methanol was therefore substituted by the low-boiling dichloromethane. When the condensation was complete, the reaction mixture was filtered, concentrated and the reduction was performed after the addition of methanol. From 380 mg (3.19 mmol) of **13**, 508 mg (3.38 mmol) of phenylglyoxylic acid, 225 mg (3.57 mmol) of $NaCNBH_3$ and 701 mg (3.19 mmol) of $Zn(OAc)_2{\cdot}2H_2O$, **4a** was obtained as a white solid in an overall yield of 161 mg (0.67 mmol, 21%). Mp = 118 °C (decomp.). ^{1}H NMR, ^{13}C NMR and MS were identical to those of **4a** obtained by starting from **14**.

4b. The synthesis was performed using 408 mg (2.09 mmol) of (*R*)-*N*-hydroxy phenyl alanine methyl ester, 202 mg (2.19 mmol) of glyoxylic acid·H_2O, 144 mg (2.29 mmol) of $NaCNBH_3$ and 459 mg (2.09 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. The conditions applied for the hydrolysis were reflux for 1 hour in 15 mL of 1 M HCl. After evaporation of the aqueous HCl, the mixture was dissolved in water and the pH was then adjusted to 1.80 with aqueous NH_4OH after which the mixture was concentrated. After precipitation with $Zn(OAc)_2{\cdot}2H_2O$, **4b** was obtained as a white solid in an overall yield of 206 mg (0.86 mmol, 41%). Mp = 130 °C (decomp.). $[\alpha]_D^{20}$ = –5.0 ° (c=1, EtOH). ^{1}H NMR (DMSO, 300 MHz, 25 °C) δ; 2.92–2.97 (m,

2H, C<u>H</u>$_2$C$_6$H$_5$,), 3.60–3.66 (m, 3H, NC<u>H</u> + NC<u>H</u>$_2$), 7.16–7.29 (m, 5H, C$_6$<u>H</u>$_5$). ^{13}C NMR (DMSO, 75 MHz, 25 °C): δ 172.51, 171.69 (2C, 2 x <u>C</u>OOH), 139.04, 129.92, 128.81, 126.83 (6C, <u>C</u>$_6$H$_5$), 71.56 (1C, N<u>C</u>H), 59.04 (1C, N<u>C</u>H$_2$), 35.73 (1C, <u>C</u>H$_2$C$_6$H$_5$). HRMS-ESI calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$ 240.0867, found 240.0864.

Synthesis of *rac*-5a and *rac*-5b. The *N*-hydroxy amino acid ester and the α-keto acid were stirred in methanol until TLC (PE/EtOAc 1:1) showed full conversion of the *N*-hydroxy amino acid ester. After the addition of $NaCNBH_3$, TLC showed a complete and immediate conversion of the nitrone (Rf <0.05; strong UV absorbtion). Evaporation of the methanol yielded a white solid, which was then dissolved in 5 mL of water. To remove the cyanide, 1–2 equivalents of aqueous HCl were added, followed by stirring of the open vessel at 60 °C in the fumehood for 20 minutes. The pH of the mixture was brought to 1.9 using NH_4OH solution, it was concentrated and left to crystallise at 0 °C after the addition of a small amount of ethanol.

Synthesis of 5a. From 580 mg (4.87 mmol) of *N*-hydroxy alanine methyl ester, 455 mg (5.16 mmol) of pyruvic acid and 343 mg (5.45 mmol) of $NaCNBH_3$, 330 mg (2.13 mmol, 44%) of **5a** was otained as white crystals. Mp = 137–138 °C (decomp.). ^{1}H NMR (H_2O, 300 MHz, 25 °C) δ; 1.34, 1.35 (2 x d, 6H, 2 x CHC<u>H</u>$_3$, *J* = 6.9), 3.74 (s, 3H, OC<u>H</u>$_3$), 3.87, 4.01 (2 x q, 2H, 2 x C<u>H</u>CH$_3$, *J* = 6.9). ^{13}C NMR (H_2O, 75 MHz, 25 °C) δ; 176.00, 174.01 (2C, 2 x <u>C</u>OOH + <u>C</u>OOMe), 62.28, 61.88 (2C, 2 x N<u>C</u>H), 52.73 (1C, O<u>C</u>H$_3$), 14.51, 14.09 (2C, 2 x N<u>C</u>H$_3$). HRMS-ESI calcd. for $C_7H_{14}NO_5$ $[M+H]^+$ 192.0867, found 192.0863. HRMS-ESI calcd. for $C_7H_{13}NO_5Na$ $[M+Na]^+$ 214.0686, found 214.0680.

Synthesis of 5b. From 130 mg (0.72 mmol) of *N*-hydroxy phenylglycine methyl ester, 66 mg (0.75 mmol) of pyruvic acid and 50 mg (0.79 mmol) of $NaCNBH_3$, 58 mg (0.23 mmol, 32%) of **5b** was obtained as white crystals. Mp = 128–130 °C (decomp.). ^{1}H NMR (DMSO, 400 MHz, 25 °C) δ; 1.13 (d, 3H, CHC<u>H</u>$_3$, *J* = 7.2), 3.07 (q, 1H, C<u>H</u>CH$_3$, *J* = 7.2), 3.58 (s, 3H, OC<u>H</u>$_3$), 5.13 (s, 1H, C<u>H</u>C$_6$H$_5$), 7.35–7.46 (m, 3H, C$_6$<u>H</u>$_5$), 8.56 (s), 12.40 (br), (2H, NO<u>H</u> + COO<u>H</u>), ^{13}C NMR (DMSO, 100 MHz, 25 °C): δ 172.95, 171.89 (2C, <u>C</u>OOMe + <u>C</u>OOH), 136.08, 129.47, 129.40 (6C, <u>C</u>$_6$H$_5$), 73.11 (1C, <u>C</u>HC$_6$H$_5$), 59.03 (1C, <u>C</u>HCH$_3$), 52.39 (1C, O<u>C</u>H$_3$), 16.76 (1C, CH<u>C</u>H$_3$). HRMS-ESI calcd. for $C_{12}H_{16}NO_5$ $[M+H]^+$ 254.1023, found 254.1020.

Synthesis of *meso*-1. A solution of 146 mg (0.76 mmol) of *rac*-**5** in 3 mL of 1M HCl was refluxed for 1 hour. The pH of the mixture was brought to 4.2 with 0.5 M NaOH solution and the product was precipitated with 168 mg (0.76 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. Compound *meso*-**1** was released by ion exchange chromatography following the procedures reported above for **1** and was isolated as a white solid in a yield of 48 mg (0.27 mmol, 35%). ^{1}H and ^{13}C NMR showed the presence of one diastereomer, which was identified as the *meso*-form of **1** after comparison with the NMR data of **1** obtained via the substitution on (*R*)-2-bromopropionate as described above. ^{1}H NMR (D_2O, 400 MHz) δ = 1.43 (d, 6H, CHC<u>H</u>$_3$, *J* = 6.0), 4.08 (q, 1H, C<u>H</u>CH$_3$, *J* = 6.0). ^{13}C NMR (D_2O, 100 MHz) δ = 175.91 (2C, <u>C</u>OOH), 64.58 (2C, <u>C</u>H), 14.76 (2C, <u>C</u>H$_3$).

Synthesis of 4a via hydrolysis of 5b. A solution of 190 mg (0.75 mmol) of compound **5b** in 4 mL 0.5 M of NaOH was stirred at RT during 6 hours. The pH of the mixture was brought to 3.8 with 1 M NaOH solution and the product was precipitated with 165 mg (0.75 mmol) of $Zn(OAc)_2{\cdot}2H_2O$. Compound **4a** was released by ion exchange chromatography following the procedures reported above for **4a** and it was isolated as a white solid in a yield of 65 mg (0.27 mmol, 36%). According to ^{1}H NMR, **4a** was present as one diastereomer. Mp = 115 °C (decomp.). ^{1}H NMR (DMSO, 300 MHz, 25 °C) δ; 1.13 (d, 3H, CHC<u>H</u>$_3$, J = 6.9), 3.09 (q, 1H, C<u>H</u>CH$_3$, J = 6.9), 4.99 (s, 1H, C<u>H</u>C$_6$H$_5$), 7.33–7.50 (m, 5H, C$_6$<u>H</u>$_5$). ^{13}C NMR (DMSO, 75 MHz, 25 °C): δ 173.12, 172.83 (2C, 2 x <u>C</u>OOH), 136.84, 129.47, 129.21, 129.09 (6C, <u>C</u>$_6$H$_5$), 73.23 (1C, <u>C</u>HC$_6$H$_5$), 59.11 (1C, <u>C</u>HCH$_3$), 16.66 (1C, CH<u>C</u>H$_3$).^{1}H NMR and ^{13}C NMR were identical to those of isomer B of **4a** as reported above.

Synthesis of secondary hydroxylamines 17a and 17c. To a solution of the nitrone **7** in 20 mL of methanol were added $NaCNBH_3$ and acetic acid, both in a 1.1 equivalent amount. The reaction was complete after 24 hours (TLC). The solvent was then evaporated, saturated aqueous $NaHCO_3$ was added, the product was extracted with 3 x 20 mL of CH_2Cl_2 and dried over $MgSO_4$.

17a. From 574 mg (2.59 mmol) of nitrone **7a**, 179 mg (2.85 mmol) of $NaCNBH_3$ and 171 mg (2.85 mmol) of acetic acid, 557 mg (2.49 mmol, 96%) of **17a** was obtained as a colourless oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.29 (t, 3H, CH$_2$C<u>H</u>$_3$, *J* = 7.2), 1.41 (d, 3H, CHC<u>H</u>$_3$, *J* = 6.9), 3.55 (q, 2H, C<u>H</u>$_2$CH$_3$, *J* = 7.0), 3.86 (1H, NC<u>H</u>$_2$, *J* = 13.2), 3.99 (1H, NC<u>H</u>$_2$, *J* = 13.2), 4.20 (q, 1H, C<u>H</u>CH$_3$, *J* = 7.2), 5.75 (s, 1H, NO<u>H</u>), 7.26–7.36 (m, 5H, C$_6$<u>H</u>$_5$), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 173.09 (1C, <u>C</u>OOEt), 137.23, 129.48, 128.33, 127. 44 (6C, <u>C</u>$_6$H$_5$), 63.87, 60.80, 60.71 (3C, N<u>C</u>H + N<u>C</u>H$_2$ + O<u>C</u>H$_2$CH$_3$), 14.22, 14.12 (2C, OCH$_2$<u>C</u>H$_3$ + CH<u>C</u>H$_3$). HRMS-ESI calcd. for $C_{12}H_{18}NO_3$ $[M+H]^+$ 224.1282, found 224.1277.

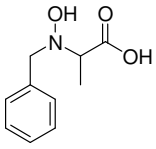
17c. From 574 mg (2.77 mmol) of nitrone **7c**, 191 mg (3.05 mmol) of $NaCNBH_3$ and 183 mg (3.05 mmol) of acetic acid, 531 mg (2.54 mmol, 92%) of **17c** was obtained as white crystals. Mp = 73 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.26 (t, 3H, CH$_2$C<u>H</u>$_3$, *J* = 7.1), 3.50 (s, 2H, NC<u>H</u>$_2$COOEt), 3.92 (s, 2H, NC<u>H</u>$_2$C$_6$H$_5$), 4.18 (q, 1H, C<u>H</u>$_2$CH$_3$, *J* = 7.1), 6.49 (s, 1H, NO<u>H</u>), 7.25–7.38 (m, 5H, C$_6$<u>H</u>$_5$), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 170.03 (1C, <u>C</u>OOEt), 136.38, 129.68, 128.39, 127.66 (6C, <u>C</u>$_6$H$_5$), 63.86, 60.85, 60.11 (3C, N<u>C</u>H$_2$C$_6$H$_5$ + N<u>C</u>H$_2$COOEt + O<u>C</u>H$_2$CH$_3$), 14.16 (1C, OCH$_2$<u>C</u>H$_3$). Anal. calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 62.95; H, 7.16; N, 6.48. HRMS-ESI calcd. for $C_{11}H_{16}NO_3$ $[M+H]^+$ 210.1125, found 210.1121.

Synthesis of secondary hydroxylamine 17d. Under a nitrogen atmosphere and at 0 °C, 385 mg (3.23 mmol) of **13**, 157 mg (3.55 mmol) of acetaldehyde and 213 mg (3.55 mmol) of acetic acid were stirred in 20 mL of CH_2Cl_2 using Na_2SO_4 as a drying agent. When TLC (PE/EtOAc 1:1) showed full conversion of the ester to the intermediate nitrone (Rf <0.05; strong UV absorbtion), 223 mg (3.55 mmol) of $NaCNBH_3$ was added. The reaction was complete after 1 hour (TLC). The solvent was then evaporated, saturated aqueous $NaHCO_3$ was added, the product was

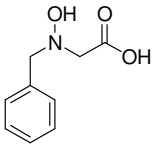
extracted with 3 x 20 mL of CH_2Cl_2 and dried over $MgSO_4$. Compound **17d** was isolated in a yield of 385 mg (2.62 mmol, 81%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.18 (t, 3H, $NCH_2C\underline{H}_3$, *J* = 7.0), 1.36 (d, 3H, $CHC\underline{H}_3$, *J* = 6.9), 2.70 (dq, 1H, $NC\underline{H}_2CH_3$), 2.88 (dq, 1H, $NC\underline{H}_2CH_3$), 3.56 (q, 1H, $NC\underline{H}CH_3$, *J* = 7.0) 3.75 (s, 3H, $OC\underline{H}_3$), 5.91 (br, 1H, $NO\underline{H}$), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 173. 73 (1C, $\underline{C}OOMe$), 64.81 (1C, $N\underline{C}H$), 51.91, 50.60 (2C, $O\underline{C}H_3$, + $N\underline{C}H_2$), 14.02, 12.16 (2C, $CH\underline{C}H_3$ + $CH_2\underline{C}H_3$). HRMS-ESI calcd. for $C_6H_{14}NO_3$ $[M+H]^+$ 148.0969, found 148.0966.

Synthesis of secondary hydroxylamines 6a, 6c and 6d. A solution of the appropriate methyl ester (**17a**, **17c**, **17d**) in 20 mL of 1M HCl was stirred at 125 °C for 1 hour, the reaction was followed with TLC (PE/EtOAc 4:1). The solvent was then evaporated and the residue was stripped twice with water to remove residual HCl. After dissolving the residue in 10 mL of water, the pH of the solution was brought to 2.4 using NH_4OH solution. The solution was concentrated and left to crystallize at 0 °C after the addition of a small amount of ethanol.

6a. The hydrolysis required 3 M HCl instead of 1 M. From 465 mg (2.08 mmol) of **17a**, 189 mg (1.04 mmol, 50%) of **6a** was obtained as white crystals. Mp = 147 °C (decomp.). ^{1}H NMR (D_2O, 300 MHz, 25 °C): 1.53 (d, 3H, $CHC\underline{H}_3$, *J* = 7.2), 3.91 (q, 1H, $C\underline{H}CH_3$, *J* = 7.1), 4.46 (s, 2H, $NC\underline{H}_2$) 7.43–7.51 (m, 5H, $C_6\underline{H}_5$), ^{13}C NMR (D_2O, 75 MHz, 25 °C): δ 175.59 (1C, $\underline{C}OOH$), 132.89, 131.49, 130.86, 130.57 (6C, $\underline{C}_6H_5$), 68.23, 62.46 (2C, $N\underline{C}H$ + $N\underline{C}H_2$), 14.17 (1C, $CH\underline{C}H_3$). HRMS-ESI calcd. for $C_{10}H_{14}NO_3$ $[M+H]^+$ 196.0969, found 196.0967.

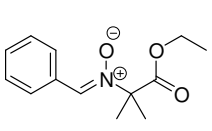


6c. From 209 mg (1.00 mmol) of **17c**, 150 mg (0.83 mmol, 83%) of **6c** was obtained as white crystals. Mp = 135 °C (decomp.). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 3.74 (s, 2H, $NC\underline{H}_2C_6H_5$), 4.35 (s, 2H, $NC\underline{H}_2COOH$), 7.33–7.38, 7.47–7.51 (m, 5H, $C_6\underline{H}_5$), 12.47 (s, 2H, $COO\underline{H}$ + $NO\underline{H}$), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 171.09 (1C, $\underline{C}OOH$), 131.62, 129.70, 129.54, 128.62 (6C, $\underline{C}_6H_5$), 62.84, 60.14 (2C, $N\underline{C}H_2C_6H_5$ + $N\underline{C}H_2COOH$). HRMS-ESI calcd. for $C_9H_{12}NO_3$ $[M+H]^+$ 182.0812, found 182.0811.



6d. The hydrolysis required 1.5 hours instead of 1 hour. When crystals could not be obtained (oily precipitate), the solution was evaporated to dryness and the raw product was then extracted with THF. The product could now be crystallized from a water/ethanol mixture. From 335 mg (2.28 mmol) of **17d**, 140 mg (1.05 mmol, 46%) of **6d** was obtained as white crystals. Mp = 146 °C (decomp.). ^{1}H NMR (DMSO, 300 MHz, 25 °C): δ 1.03 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 1.17 (d, 3H, $CHC\underline{H}_3$, *J* = 6.6), 2.61 (dq, 1H, $NC\underline{H}_2CH_3$), 2.71 (dq, 1H, $NC\underline{H}_2CH_3$), 3.29 (q, 1H, $C\underline{H}CH_3$, *J* = 6.6), 7–12 (br, 2H, $COO\underline{H}$ + $NO\underline{H}$), ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 174.40 (1C, $\underline{C}OOH$), 65.61 (1C, $N\underline{C}HCH_3$), 51.02 (1C, $N\underline{C}H_2$), 14.76, 13.07 (2C, $CH\underline{C}H_3$ + $CH_2\underline{C}H_3$). HRMS-ESI calcd. for $C_5H_{12}NO_3$ $[M+H]^+$ 134.0812, found 134.0811.

Synthesis of nitrone 7d. The synthesis was performed accoring to the literature procedure with few modications. Under a nitrogen atmosphere, 0.801 g (7.14 mmol) of KO*t*-Bu was suspended in 15 mL of THF. The mixture was stirred for 10 minutes at RT and then for 10 minutes at –70 °C. A suspension of 1.549 g (7 mmol) of nitrone **7a** in 5 mL of THF was slowly added and the resulting mixture was stirred for 20 minutes, the temperature being



maintained at –70 °C. A solution of 1.033 g (7.28 mmol) of MeI in 5 mL of THF was then added and the mixture was stirred for 18 hours during which it was allowed to warm up to RT. As indicated by wet pH-paper, the pH of the solution had decreased to almost neutral. The solvent was removed by rotary evaporation and 30 mL of water was added to the resulting sticky yellow solid. The product was extrated with 4 x 30 mL of CH_2Cl_2, dried on Na_2SO_4 and the resulting oil was left to become a solid at –18 °C. Washing of the solid with PE/Et_2O 5/1 yielded 1.248 g (5.30 mmol, 76%) of **7d** as a white solid. Mp = 63–64 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.27 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.2), 1.83 (s, 6H, $NC(C\underline{H}_3)_2$), 4.26 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.1), 7.42 (m, 3H, $C_6\underline{H}_5$), 7.48 (s, 1H, N=C<u>H</u>), 8.27 (m, 2H, $C_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 170.50 (1C, <u>C</u>OOEt), 131.17 (1C, N=<u>C</u>H), 130.60, 130.37, 128.99, 128.50 (6C, $\underline{C}_6H_5$), 62.10 (1C, $O\underline{C}H_2CH_3$), 24.50 (1C, $\underline{C}(CH_3)_2$), 13.97 (1C, $OCH_2\underline{C}H_3$). HRMS-ESI calcd. for $C_{13}H_{18}NO_3$ $[M+H]^+$ 236.1282, found 236.1279. HRMS-ESI calcd. for $C_{13}H_{17}NO_3Na$ $[M+H]^+$ 258.1101, found 258.1098.

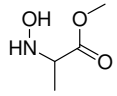
Synthesis of 16. To a solution of 0.780 g (3.32 mmol) of nitrone **7d** in methanol was added 0.242 g (3.48 mmol) of hydroxylamine hydrochloride. After TLC (PE/EtOAc 1:1) showed full conversion, two drops of 6 M HCl were added, the solvent was evaporated and the residue was stripped with diethyl ether. The HCl salt of **16** obtained crystallized quickly from a 1 : 1 diethyl ether-petrolether mixture and was isolated in a yield of 0.586 g (3.19 mmol, 96%), NMR conforming complete removal of the benzaldoxime co-product. To an aqueous solution of the crystals was added saturated $NaHCO_3$ solution. After evaporation of the water, the residue was suspended in diethyl ether and dried on Na_2SO_4. After filtration, **16** was obtained in a yield of 382 mg (2.60 mmol, 78%) as a colourless oil. This is the only *N*-hydroxy amino acid ester that was not visible using ninhydrin, but it could be identified using iodine. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.29 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.1), 1.32 (s, 6H, $NC(C\underline{H}_3)_2$), 4.21 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.2), 5.70, 6.53 (br, 2H, N<u>H</u>O<u>H</u>). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 175.59 (1C, <u>C</u>OOEt), 62.81, 61.23 (2C, $\underline{C}(CH_3)_2$ + $O\underline{C}H_2CH_3$), 22.14 (2C, $C(\underline{C}H_3)_2$), 14.17 (1C, $OCH_2\underline{C}H_3$). HRMS-ESI calcd. for $C_6H_{14}NO_3$ $[M+H]^+$ 148.0969, found 148.0967.

Synthesis of 12. Prepared according to the literature procedure with few modifications.[74] To a solution of 96 mg (4.16 mmol) of Na in 20 mL of methanol were added 289 mg (4.16 mmol) of hydroxylamine hydrochloride and 750 mg (3.62 mmol) of nitrone **7c**. After TLC (PE/EtOAc 1:1) showed full conversion, the solvent was evaporated cautiously (volatile product). The remaining oil was purified by flash column chromatography over a 10 x 5 cm silica plug using PE/EtOAc 1:1. Compound **12** was then obtained in a yield of 400 mg (3.36 mmol, 93%) as a pale yellow solid. Mp = 42–44 °C. ^{1}H NMR (CH_2Cl_2, 300 MHz, 25 °C) δ; 1.28 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.2), 3.66 (s, 2H, $NC\underline{H}_2$), 4.22 (q, 2H, $C\underline{H}_2CH_3$, *J* = 7.2), 6.11 (s, 2H, N<u>H</u>O<u>H</u>). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 171.29 (1C, <u>C</u>OOEt), 61.41 (1C, $O\underline{C}H_2$), 55.10 (1C, $N\underline{C}H_2$), 14.38 (3C, $CH_2\underline{C}H_3$).

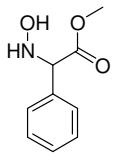
Synthesis of *N*-hydroxy amino acid methyl esters 13 and 14 via the 'acetaldoxime route'. Under a nitrogen atmosphere, the sodium was dissolved in 50 mL of dry methanol. After the addition of the

acetaldoxime and the appropriate α-bromo methyl ester, the mixture was stirred overnight. By then, the pH of the solution had decreased to almost neutral, as indicated by wet pH-paper. After the addition of the hydroxylamine hydrochloride, TLC (PE/EtOAc 1:1) showed a complete and immediate conversion of the nitrone. The solvent was evaporated, the residue was dissolved in 30 mL of 1 M HCl and was then washed with 5 x 30 mL of diethyl ether. To the aqueous layer was added saturated $NaHCO_3$ solution and the product was then extracted with diethyl ether until TLC could not detect the product in the water layer anymore. The combined layers were dried over Na_2SO_4, after which the *N*-hydroxy amino acid methyl ester was isolated.

13. Because of the high solubility of the product in water, the extraction with diethyl ether after the $NaHCO_3$ addition was difficult. Instead, the water was evaporated, the residue was suspended in diethyl ether and dried over Na_2SO_4. From 19.674 g (117.80 mmol) of methyl 2-bromopropionate, 6.627 g (112.19 mmol) of acetaldoxime, 2.579 g (112.19 mmol) of sodium and 3.898 g (56.10 mmol) of hydroxylamine hydrochloride, 4.174 g (35.04 mmol, 31%) of **13** was obtained as white crystals. The analytical data matched those reported in the literature.[69] Mp = 29–30 °C. ^{1}H NMR (H_2O, 300 MHz, 25 °C) δ; 1.27 (d, 3H, CHC<u>H</u>$_3$, *J* = 7.2), 3.75 (q, 1H, C<u>H</u>CH$_3$, *J* = 7.2), 3.77 (s, 3H, OC<u>H</u>$_3$), 6.04 (br, 2H, N<u>H</u>O<u>H</u>).



14. From 5.249 g (22.91 mmol) of methyl 2-bromo-2-phenylacetate, 1.301 g (22.03 mmol) of acetaldoxime, 0.507 g (22.03 mmol) of sodium and 1.531 g (22.03 mmol) of hydroxylamine hydrochloride, 1.275 g (7.04 mmol, 32%) of **14** was obtained as a white solid. The analytical data matched those reported in the literature.[75] Mp = 71 °C. ^{1}H NMR (H_2O, 300 MHz, 25 °C) δ; 3.75 (s, 3H, OC<u>H</u>$_3$), 4.78 (s, 1H, C<u>H</u>C$_6$H$_5$), 5.89 (br, 2H, N<u>H</u>O<u>H</u>), 7.34 (s, 5H, C$_6$<u>H</u>$_5$).



Synthesis of (*R*)-*N*-hydroxy phenylalanine methyl ester 15. The three-step synthesis was performed according to the literature procedure[69] with few modifications. <u>1. Synthesis of the imine.</u> To a 25 mL aqueous solution of 5.39 g (24.99 mmol) of (*R*)-phenylalanine methyl ester hydrochloride was added an aqueous solution of 2.91 g (27.49 mmol) of Na_2CO_3. Extraction with 4 x 15 mL of dichloromethane, drying on Na_2SO_4 and evaporation of all solvents yielded the crude phenyl alanine methyl ester, which was then condensed with anisaldehyde using a Dean-Stark trap with toluene. Evaporation of the toluene yielded the crude imine in a yield of 7.356 g (24.740 mmol, 99%). ^{1}H NMR showed traces of the phenylalanine methyl ester and the aldehyde, but it was clean enough for the next step. <u>2. synthesis of the oxaziridine.</u> The imine (6.31 g, 21.22 mmol) was dissolved in 40 mL of DCM, cooled to -8 ^{o}C and a solution of 6.34 g (21.22 mmol) of MCPBA (77 %) in 60 mL of DCM was added. The mixture was allowed to reach RT overnight, and a white precipitate was formed in a yellow solution. The mixture was filtered, washed with 25 mL of bicarbonate and 25 mL of brine. After drying on $MgSO_4$, a clear brown oil was obtained. <u>3. synthesis of the *N*-hydroxy phenylalanine methyl ester.</u> The crude oxaziridine (5.66 g, 18.05 mmol) was dissolved in 50 mL of MeOH and 1.25 g (18.05 mmol) of H_2NO<u>H</u> was added. After 30 minutes, all starting material was converted (TLC) and the mixture was left to stir overnight. All volatiles were evaporated, 25 mL of water was added, and this acidic solution (checked with pH indicator) was washed with 2 x 25 mL of Et_2O. Bicarbonate solution was added until the pH was 8 and then the mixture was extracted with 3 x

25 mL of diethyl ether. The collected diethyl ether layers were dried on Na_2SO_4 and the Et_2O was removed under reduced pressure, yielding a light-brown oil. Purification over a 5 x 10 cm silica plug with EtOAc / PE = 1 / 1 yielded the product in 1.158 g (5.932 mmol, 27% starting from (*R*)-phenylalanine methyl ester hydrochloride). Mp: 62–64 °C. $[\alpha]_D^{20} = -16.4°$ (c=1, C_6H_6). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ; 2.94 (m, 2H, C<u>H</u>$_2$C$_6$H$_5$), 3.72 (s, 3H, OC<u>H</u>$_3$), 3.88 (m, 1H, C<u>H</u>CH$_2$), 5.46 and 6.16 (br, 2H, N<u>H</u>O<u>H</u>) 7.17-7.32 (m, 5H, C$_6$<u>H</u>$_5$). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 173.28 (1C, <u>C</u>OOMe), 136.46, 129.00, 128.63, 126.97 (6C, <u>C</u>$_6$H$_5$), 66.14 (1C, N<u>C</u>H$_2$), 52.04 (1C, OC<u>H</u>$_3$) 35.44 (1C, <u>C</u>H$_2$C$_6$H$_5$).

3.7. Acknowledgements

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3.8. References

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COMPLEXATION OF VANADIUM BY AMAVADIN-BASED LIGANDS

4

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4.1. Introduction

4.1.1. Importance of the N-hydroxy group for the ligand-vanadium binding in amavadin

Following the identification of the ligand of amavadin in 1973[1] as *N*-hydroxyimino-(2,2')-dipropionic acid **1** (Figure 4.1), it was not until 1983[2] that further studies were initiated towards the nature of this ligand and its interaction with transition metals. The *N*-hydroxy group is the most remarkable feature of the ligand and the investigations were focused on this moiety.[3,4,5,6] For this purpose, the natural ligand **1** and the achiral minimal ligand **2** were investigated. Furthermore, the imino-dicarboxylic acids **3** and **4** which lack the *N*-hydroxy group were included in the study.[7] The latter compounds were already known as bidentate ligands in their complexation of transition metals.

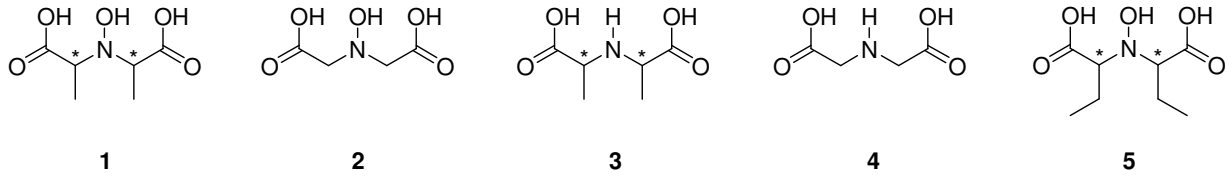


Figure 4.1. *Amavadin and related ligands that were used for the calculation of stability constants.*

Quantitative investigations of the effect of *N*-hydroxy group were performed[3,5] by measuring the stability constants $K_n = [ML_{n-1}][L]/[ML_n]$ of complexes of ligands (L) **1**–**4** with the metal ions (M) Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, VO^{2+} and Al^{3+}. For all metals except vanadium the values of K_1 and K_2 show the expected trends. Only for vanadium in combination with the *N*-hydroxy compounds **1** or **2**, exceptionally high stability constants were found, being 10 orders of magnitude larger than for the other complexes. In addition, **1** and **2** gave only VL_2 and no VL complexes. As a result, only an overall stability constant β_2 could be measured, leading to the unusual stability sequence of $K_1 < K_2$. This is even more remarkable because ligands **3** and **4** give ML_2 complexes with all metals, but with vanadium only ML complexes are observed. Another study on the effect of the *N*-hydroxy group in the ligand dealt with the electrochemistry of amavadin.[6] Reversible one-electron oxidation couples were only observed for vanadium complexes consisting of ligands **1** and **2**, whereas vanadium complexes of **3** and **4** show irreversible oxidations. This suggests that amavadin has the role of an electron transfer catalyst in the mushroom. From these studies it thus became evident that the *N*-hydroxy group plays a very important role in amavadin. Apparently, it had a direct interaction with the vanadium and, more specifically, it was responsible for the very high selectivity of the ligand towards vanadium. These findings shed light on the function and the formation of amavadin in nature, but raised questions regarding the supposed vanadyl-structure of amavadin (Figure 4.2).

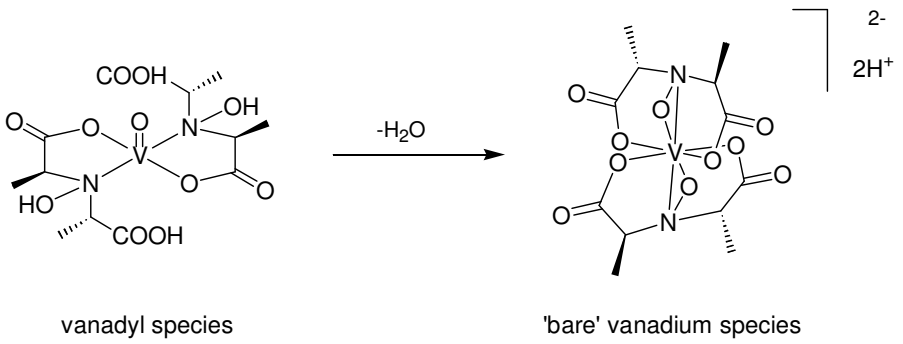
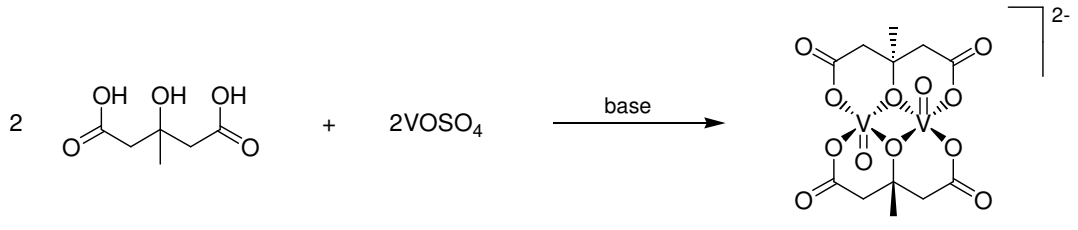


Figure 4.2. *The initially proposed vanadyl species and the actual octahedral amavadin structure.*

Oxo ligands predominate in the chemistry of vanadium in its +4 and +5 oxidation states and the vanadyl ion ranks among the most stable of diatomic cations. However, the stability constants of the vanadium complexes are so high that they are improbable for 1:2 pentacoordinated oxovanadium(IV) complexes. On this basis, it was postulated in 1987 by Bayer and coworkers[5] that amavadin exists as an octahedral vanadium non-oxo compound, a so-called 'bare' vanadium species. The original oxo-ligand is expelled from the vanadium as one molecule of water, while the *N*-hydroxy group binds side-on to the vanadium. In 1999,[8] this was confirmed by X-ray studies on samples of amavadin crystallized as a phosphoric acid derivative or as the Ca^{2+} salt. The coordination sphere of vanadium involves four unidentate carboxylate groups and two η^2-NO groups and the complex is indeed octahedral (considering the N-O groups as one corner). Furthermore, the two ligands are connected to the vanadium in a meridional fashion.

This structure elucidation inspired others to investigate the vanadium coordination chemistry of another amavadin ligand analogue: the 3-hydroxy-3-methylglutarate ligand **6**.[9] In this analogue, the N-OH has been changed to a C-OH moiety. An X-ray crystallographic structure of the vanadium complex **7** showed that the absence of nitrogen in the ligand results in a significantly different coordination chemistry. The ligand favors the formation of a dimeric structure and uses the deprotonated oxygen of the alcohol functional group to bridge two adjacent oxovanadium(V) centers (Scheme 4.1).

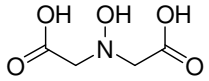
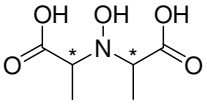
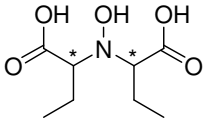


Scheme 4.1. *Complexation of 3-hydroxy-3-methylglutarate to vanadium.*

4.1.2. Early transition metal complexes of amavadin ligands

The unusual geometry and chemical behaviour of amavadin have generated curiosity as to whether it is possible to extend this chemistry to other metals. In the studies of the complexation of the amavadin ligand to other metals than vanadium as described above, no metal complexes were isolated or characterized. In 1994,[10] the group of Garner reported the first synthesis and characterisation of a metal analogue of amavadin, namely the molybdenum-substituted amavadin. Following the diagonal trend of the Periodic Table, they also treated the amavadin ligand with oxorhenium(V)[11] compounds. However, ligand rearrangement and/or fragmentation took place with this metal and the resulting rhenium complexes did not contain the amavadin ligand as such. In subsequent research by this group, the reactivity towards niobium,[12] tantalum,[13] titanium[14] and zirconium[15] was investigated. This led indeed to the corresponding ML_2-compounds, most of which were characterized by single-crystal X-ray diffraction. A complete overview of the amavadin-type metal complexes with ligands **1**, **2** and **5** that have been described in the literature is shown in Table 4.1. There appears to be a great variety in ligand and vanadium configurations of the different crystalline complexes. The two tantalum complexes with ligand **1**, for example, were obtained with either a Δ or a Λ configuration on tantalum (see below for definition), depending on the conditions of crystallisation. Although ligand **1** was only used as a mixture of diastereisomers, most of the crystallized metal complexes of **1** consisted exclusively of (*R*,*R*)- or (*S*,*S*)-ligands. This means that selective crystallisation took place with respect to the ligand stereochemistry. It was also found that the metal can change its configuration after complexation (epimerization), while the ligand is configurationally stable under the conditions of complexation and isolation. Using a slow vapour-diffusion technique, Δ or Λ forms could be selectively crystallized, indicating that a dynamic epimerisation process is proceeding in solution during the crystallisation. Moreover, it was found that epimerization takes place in fresh solutions of the $[\Delta\text{-Ti}^{IV}(R,R\text{-L})_2]^{2-}$ [14] and the $[\Delta\text{-V}^{IV}\ (S,S\text{-L})_2]^{2-}$ [16] crystalline anions. The formation of the respective Λ isomers until equilibrium has been reached could be followed using NMR. This is an example of what has been termed 'predetermination of chirality-at-metal',[17,18] which is defined as the possible preferential formation of one isomer when chiral non-racemic ligands are used.[19,20]

Table 4.1. *Overview of the amavadin-type metal complexes that have been described in the literature.*

Metal	**Ligand (LH_3)**		
	 **2**	 **1**	 **5**
Mo(V)	$[\Delta,\Lambda\text{-}MoL_2]^-$ $[PPh_4]^+ \cdot CH_2Cl_2$ [21]	$[\Delta\text{-}Mo(R,R\text{-}L)(R,S\text{-}L)]^-$ $[PPh_4]^+$ [10]	$[Mo(R,S\text{-}L)_2]^-$ $[PPh_4]^+ \cdot 2H_2O$ [21] *
		$[\Delta\text{-}Mo(R,R\text{-}L)_2]^-$ $[H_5O_2]^+$ [21]	$[\Delta,\Lambda\text{-}Mo(R,S\text{-}L)_2]^-$ $[Na]^+ \cdot ¼i\text{-}Pr_2O$ [21]
Nb(V)	$[\Delta,\Lambda\text{-}NbL_2]^-$ $[PPh_4]^+$ [12]	$[\Delta,\Lambda\text{-}Nb(R,R\text{-}L)_2]^-$ $[NEt_4]^+$ [12]	
		$[\Delta,\Lambda\text{-}Nb(R,S\text{-}L)_2]^-$ $[PPh_4]^+$ [12]	
Ta(V)	$[\Delta,\Lambda\text{-}TaL_2]^-$ $[PPh_4]^+$ [12]	$[\Lambda\text{-}Ta(R,R\text{-}L)_2]^-$ $[NEt_4]^+$ [13]	
		$[\Delta\text{-}Ta(R,R\text{-}L)_2]^-$ $[NEt_4]^+ \cdot THF$ [13]	
Ti(IV)	$[\Delta,\Lambda\text{-}TiL_2]^{2-}$ $[NMe_4]^+_2 \cdot 1½H_2O$ [14]	$[\Delta\text{-}Ti(R,R\text{-}L)_2]^{2-}$ $[NMe_4]_2^+ \cdot H_2O$ [14]	
	$[\Delta,\Lambda\text{-}TiL_2]^{2-}$ $[Ca(H_2O)_4]^{2+} \cdot 2H_2O$ [14,15]	$[\Delta\text{-}Ti(R,R\text{-}L)_2]^{2-}{}_4$ $[Ca(H_2O)_4]^{2+}$ $[Ca(H_2O)_5]^{2+}{}_3 \cdot 2H_2O$ [14]	
Zr(IV)	$[\Delta,\Lambda\text{-}ZrL_2(H_2O)]^{2-}$ $[Ca(H_2O)_5]^{2+} \cdot 2H_2O$ [15] †		
V(IV)	$[VL_2]^{2-}$ $[NH_4]^+$ $[NMe_4]^+$ [22,23] ‡	$[\Delta\text{-}V(S,S\text{-}L)_2]^{2-}$ $[Ca(H_2O)_5]^{2+} \cdot 2H_2O$ [8]	$[\Delta,\Lambda\text{-}V(R,S\text{-}L)_2]^{2-}$ $[Ca(H_2O)_2]^+ \cdot ½H_2O \cdot ½i\text{-}PrOH$ [24]
	$[\Delta,\Lambda\text{-}VL_2]^{2-}$ $[Ca(H_2O)_5]^{2+} \cdot H_2O$ [24]	$[\Lambda\text{-}V(S,S\text{-}LH)_2] \cdot H_3PO_4 \cdot H_2O$ [8]	
V(V)	$[VL_2]^-$ $[PPh_4]^+ \cdot CH_2Cl_2$ [25] ‡	$[\Delta\text{-}V(S,S\text{-}L)_2]^-$ $[PPh_4]^+ \cdot H_2O$ [25]	$[\Delta,\Lambda\text{-}V(R,S\text{-}L)_2]^-$ $[PPh_4]^+$ [24]

* This is the only complex of which no crystal structure was measured.

† With zirconium a nine-coordinate complex is formed as the size of the Zr allows the extra coordination of 1 equivalent of water.

‡ No Δ/Λ vanadium configuration was assigned to this structure.

4.1.3. Δ and Λ isomers of amavadin.

When octahedral complexes with didentate or tridentate ligands contain a chiral metal center, the chirality is denoted by Δ for a right-handed and Λ for a left-handed helix.[26,27,28] It has previously been demonstrated that amavadin is an example of such complexes[8] and that the complex is prone to epimerization in solution due to the configurationally labile vanadium atom.[16,25,29] Based on the crystallographic and NMR data of amavadin reported in the literature, we made a schematic picture of amavadin that accounts for the Δ / Λ isomerism, the complex symmetry and the epimerization (Figure 4.3).

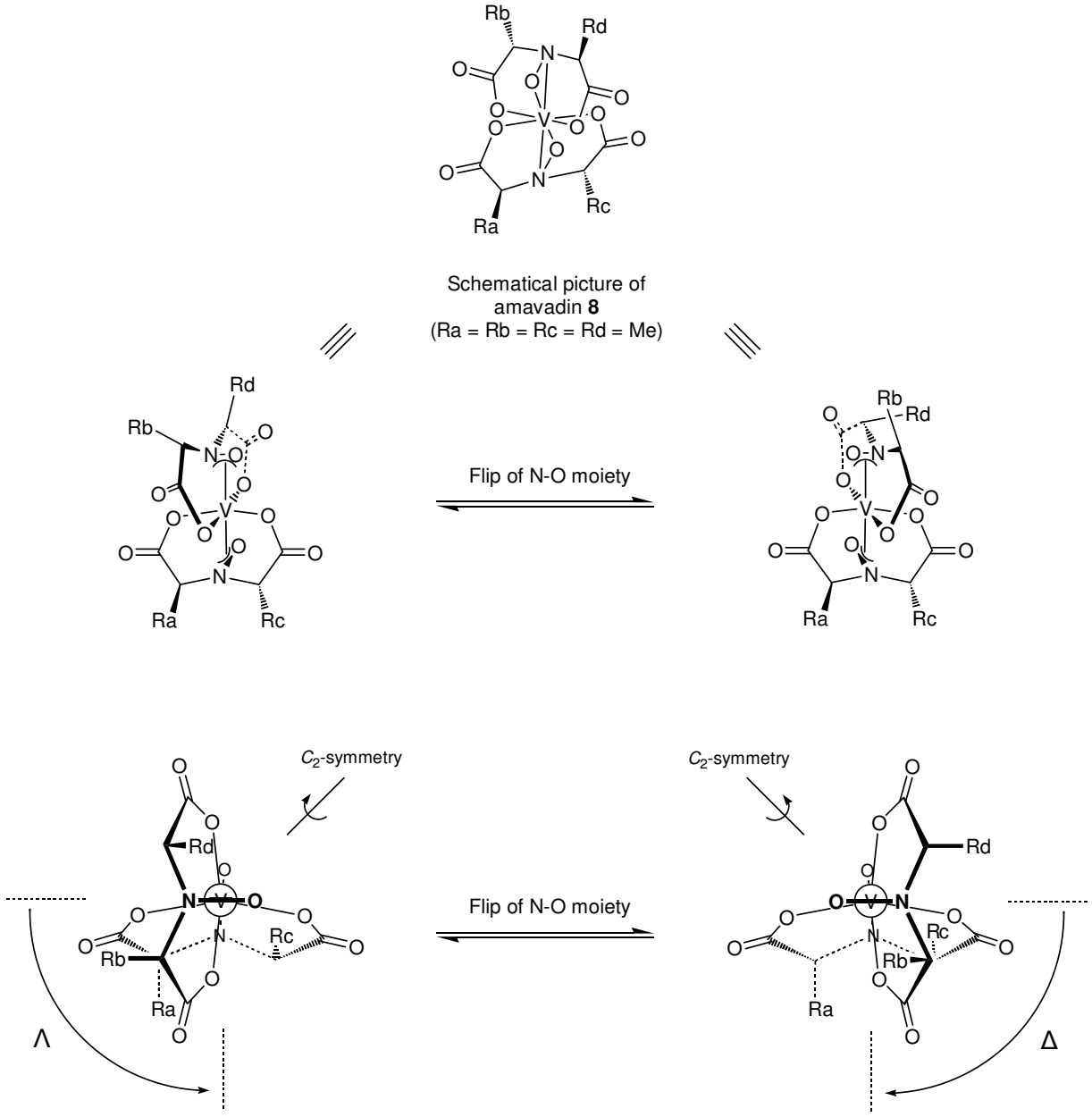
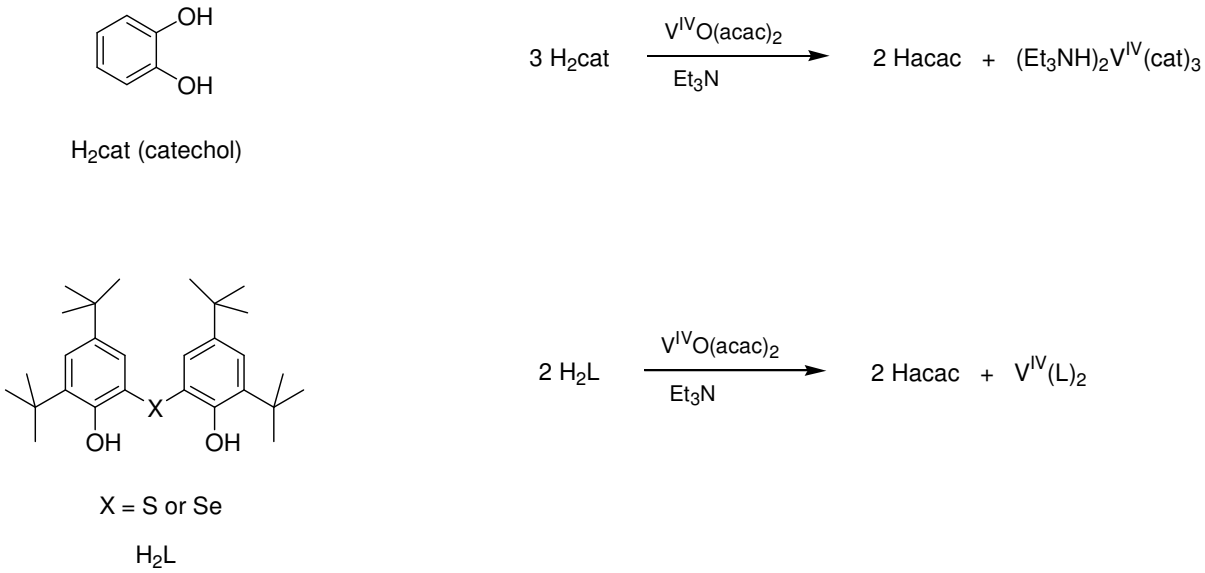


Figure 4.3. *Equatorial (VO_4-plane) and axial representations of the Λ and Δ diastereomers of amavadin that account for the the Δ / Λ isomerism, the complex symmetry and the epimerization.*

The top structure is a picture of the natural amavadin **8** (Ra = Rb = Rc = Rd = Me), representing the Δ as well as the Λ isomer. The more detailed representations below show the Δ and Λ isomers from the equatorial and the axial points of view. The interconversion between Δ and Λ diastereomers occurs via a flip of one of the N-O moieties, which results in mirrored positions of the carboxylates and the *N*-O groups while the directions of the methyl groups on the two chiral ligands are not mirrored. When viewed axially, the staggered methyl groups in the Λ isomer (Ra and Rb) point towards the equatorial direction, while the staggered methyl groups in the Δ isomer (Rb and Rc) point towards the axial direction. The axial representation also reveals C_2-symmetry with respect to an equatorial axis of rotation, which is in accordance with the observation that the four propionate units in the complex result in two different sets of signals in the ^{1}H and ^{13}C NMR of oxidized amavadin.[16,29] It has previously been shown that NMR measurements of the vanadium(V) complexes are a valid tool to discuss the properties of the vanadium(IV) complexes, as only minimal structural changes take place upon the one-electron oxidation to vanadium(V) when the amavadin ligand **1**[8,25] or its analogues **2**[22,25] and **5**[24] are complexed to vanadium.

4.1.4. Vanadium non-oxocomplexes

The amavadin ligand is one of the few ligands that produces a vanadium non-oxo compound from an oxovanadium precursor under physiological conditions. Since the discovery of the non-oxo structure of amavadin, the synthesis of other vanadium non-oxo complexes has attracted increasing interest.[30] Another natural example that has been found is the cofactor in vanadium nitrogenase (see Figure 1.3 on p. 16). However, most other examples concern synthetic complexes (Scheme 4.2).[31,32]



Scheme 4.2. *Examples of ligands that produce a vanadium non-oxo complex upon complexation.*

The use of non-oxo vanadium precursors (e.g. $V(III)Cl_3(THF)_3$) is one way to obtain vanadium non-oxo complexes, but it is more challenging to use oxovanadium as a starting material. This requires displacement of the oxo-group upon complexation, which depends not only on the proper choice of reaction conditions, but in fact mainly on the nature of the ligand. Namely, the ligand should have the ability to donate sufficient electron density (σ and π) to the metal to stabilize the 'bare species'. One of the earliest examples is the catecholate ligand, yielding the tris(catecholato)vanadium(IV)$^{2-}$ ion.[33] Later, also sulfur-substituted analogues of this ligand type have been investigated.[34] Another example includes tridentate bisphenol ligands containing [O, S, O] or [O, Se, O] donor atoms.[31] A recent review on the development of the coordination chemistry of $VO(acac)_2$ gives a concise overview of the non-oxo vanadium complexes known to date.[32]

4.1.5. Aim

This chapter describes the results obtained with the vanadium complexation of the ligands that were described in Chapter 3. The general feature of these ligands is the presence of a secondary hydroxylamine unit. Three aims were formulated, each of which will be treated in the successive sections of this chapter.

1) Section 4.2: The aim was to see how the differences in stereochemistry of the amavadin ligand are manifested in ^{1}H, ^{13}C and ^{51}V NMR spectroscopy.

2) Section 4.3: The aim was to prepare analogues of the natural amavadin that contain a phenyl or benzyl group at the backbone. Secondly, we aimed at the interpretation of their NMR-data with the aid of the model that we proposed in section 4.2.

3) Section 4.4: The aim was to investigate if the amavadin structure is retained when the backbone carries larger substituents or when the carboxylate groups are modified (see also Chapter 3: Introduction). The literature reports on the interaction of vanadium with *N*-hydroxy amino compounds are limited to the inorganic hydroxylamine ligand[35,36] (H_2NOH) and its *N*-methyl[37] and *N*,*N*-dimethyl analogues.[38,39] There are no reports on the vanadium complexation by *N*-hydroxy amino acids and derivatives thereof.

4.2. Synthesis and characterization of amavadin stereoisomers

4.2.1. NMR investigation on amavadin stereoisomers

As a result of the investigations towards new synthesis routes, the *meso*-ligand **1b** had become available in high stereochemical purity, while two mixtures of ligand isomers were isolated: (*S*,*S*)-ligand contaminated with 20% *meso*-compound (**1c**) and racemic ligand in which the *meso*-compound is present in 20% *de* (**1d**; Figure 4.4). ^{1}H, ^{13}C and ^{51}V NMR measurements

were carried out on samples of oxidized amavadin that is prepared from ligand samples **1a**–**1d**. The ^{1}H and ^{51}V NMR spectra of these samples are shown in Figure 4.5.

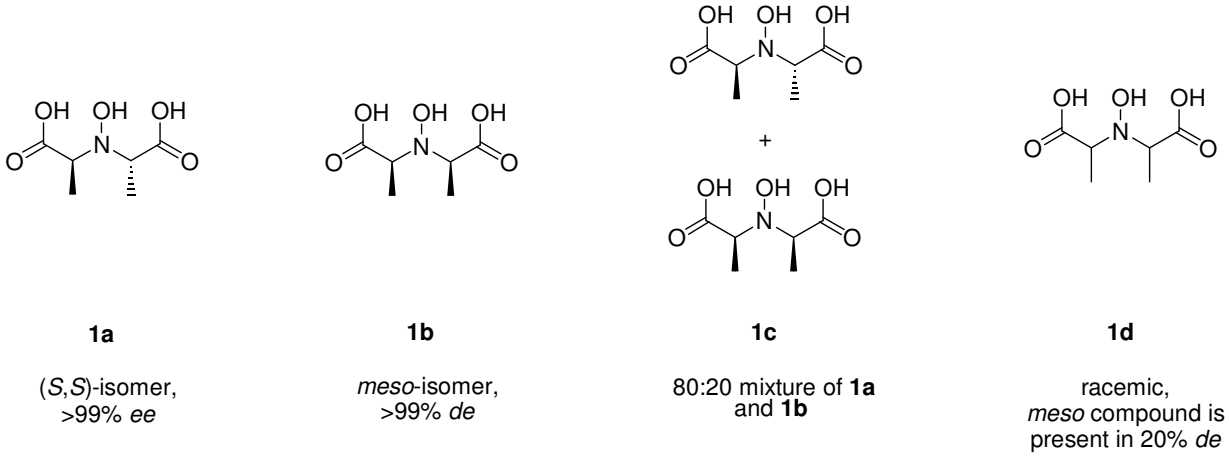


Figure 4.4. *Different amavadin ligand samples with different stereochemical composition.*

Ligand 1a. The Δ and Λ vanadium diastereoisomers could clearly be observed in the ^{1}H NMR spectrum, all the more because their ratio is not 1 to 1. Furthermore, the configurational change at the vanadium could accurately be followed as a function of time by integration of the well-separated signals of the methine protons (chapter 2). The ^{51}V NMR spectrum shows one signal, indicating that the vanadium centre itself is barely influenced by the asymmetry that it induces in the molecule.

Ligand 1b. There are two doublets and two quartets in the ^{1}H NMR[40] and six resonances in the ^{13}C NMR (two signals for each of the three propionate carbons). This proves that the complex is present as one C_2-symmetric diastereoisomer, most probably as an enantiomeric pair of Δ and Λ isomers. In section 4.2.3, the vanadium isomerism in the case of ligand **1b** is further elaborated. The ^{51}V NMR resonance has significantly shifted to lower field as compared to the complex with **1a**.

Ligand 1c. The ligand mixture **1c** was synthesized in the same way as the sample that was used by the group of Garner, which resulted in ^{1}H and ^{13}C NMR spectra that are similar to those described in the literature.[16] The ^{1}H and ^{51}V NMR clearly show the presence of other amavadin isomers besides the signals corresponding to **1a**.

Ligand 1d. The ligand mixture **1d** contained an almost statistical distribution of isomers, which is reflected by the major signals in the ^{1}H and ^{51}V NMR that correspond to amavadin with **1b** as ligands and the minor signals corresponding to amavadin with **1a** as ligands.

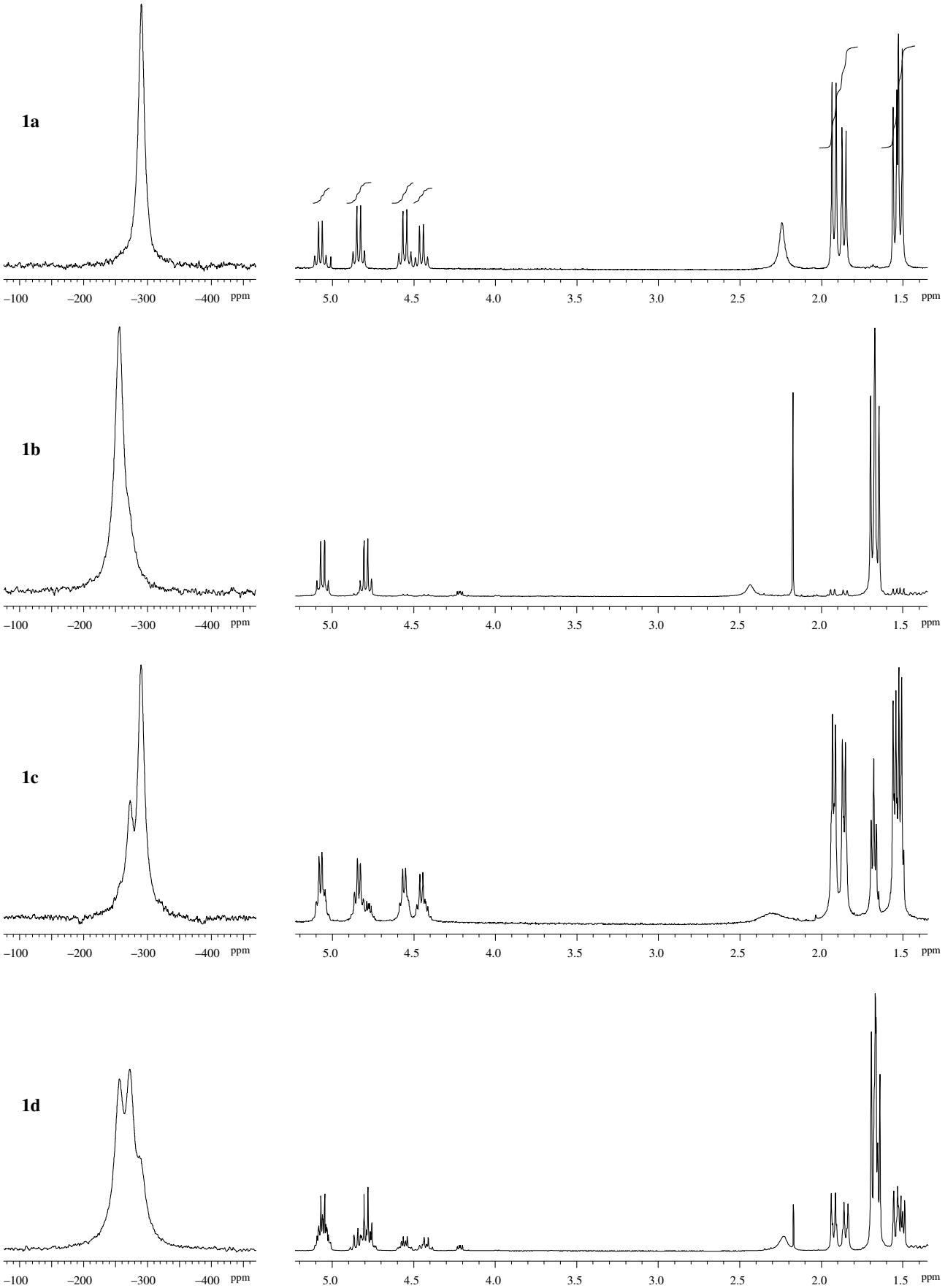


Figure 4.5. *The ^{1}H and ^{51}V NMR spectra of amavadin prepared from ligands **1a–1d** (cf. Figure 4.4).*

4.2.2. X-ray analysis of 'meso-amavadin'

Research on the structural characterization of amavadin in the 1980's was hampered by the lack of X-ray determinations. Efforts to obtain suitable single crystals of amavadin failed repeatedly[4,5] and only in 1999[8] X-ray studies were reported after the crystallisation of amavadin as its Ca^{2+} salt $[\Delta\text{-V}(S,S\text{-L})_2]^{2-}$ $[Ca(H_2O)_5]^{2+}\cdot 2H_2O$ or as the phosphoric acid adduct $[\Lambda\text{-V}(S,S\text{-LH})_2]\cdot H_3PO_4\cdot H_2O$. These derivatives are clearly different from amavadin in its diacidic form $V(S,S\text{-LH})_2$. Elucidation of the structure of the diacidic from is still interesting,[41] because the location of the protons either on the acid groups or on water molecules can have a significant influence on the structure. Questions can be posed such as "Is the octahedral vanadium surrounding also in a meridional fashion, and not facial?", "How many water molecules are enclosed?", "Is the structure consistent with the proposition[26] that the two protons are bound to two of the carboxylate groups?"

Chapter 2 reported on our efforts to obtain crystals from the diacidic form $V(S,S\text{-LH})_2$, but these were neither successful. Among the isomeric vanadium complexes prepared from ligand samples **1a**–**1d**, only the one prepared from *meso*-ligand **1b** has no precedent in the literature. Surprisingly, we found that evaporation of an aqueous solution of the so-called '*meso*-amavadin' yielded crystals immediately. Moreover, these crystals were suitable for an X-ray structure determination (Figure 4.6).

The vanadium surrounding in the crystal structure of '*meso*-amavadin' is similar to that reported earlier for the Ca^{2+} salt of the naturally occurring amavadin.[8] The four unidentate carboxylates constitute the equatorial VO_4-plane and the two N-O groups bind side-on above and below the plane. The two ligands coordinate in a meridional fashion to the octa-coordinated vanadium center. The asymmetric unit contains a vanadium complex with the composition $[VL_2H]^-$, which means that formally one of the ligands has two deprotonated carboxylate groups and the other ligand has one deprotonated and one protonated carboxylate group. A more detailed analysis suggests that each ligand is partially protonated at O8 and O2 respectively. Both atoms form a relatively short hydrogen bond with O..O = 2.503(2) Å. The associated coordinating O1 & O6 have a distance to V1 that is in the order of 0.04 Å longer than the corresponding distances for the deprotonated carboxylate groups (Figure 4.6). In addition, the asymmetric unit contains two molecules of water and a hydronium (i.e. $[H_2O\text{-}H\text{-}OH_2]^+$) cluster. An O..O distance of 2.446(2) Å is characteristic for such a species. Thus, four molecules of water are present per vanadium. Hydrogen bridges connect the asymmetric units into an infinite threedimensional network, whereby only one short hydrogen bridge forms a direct link between neighboring vanadium complexes. The unit cell contains four vanadium complexes, that are pairwise exact mirror images and linked into infinite chains (Figure 4.7).

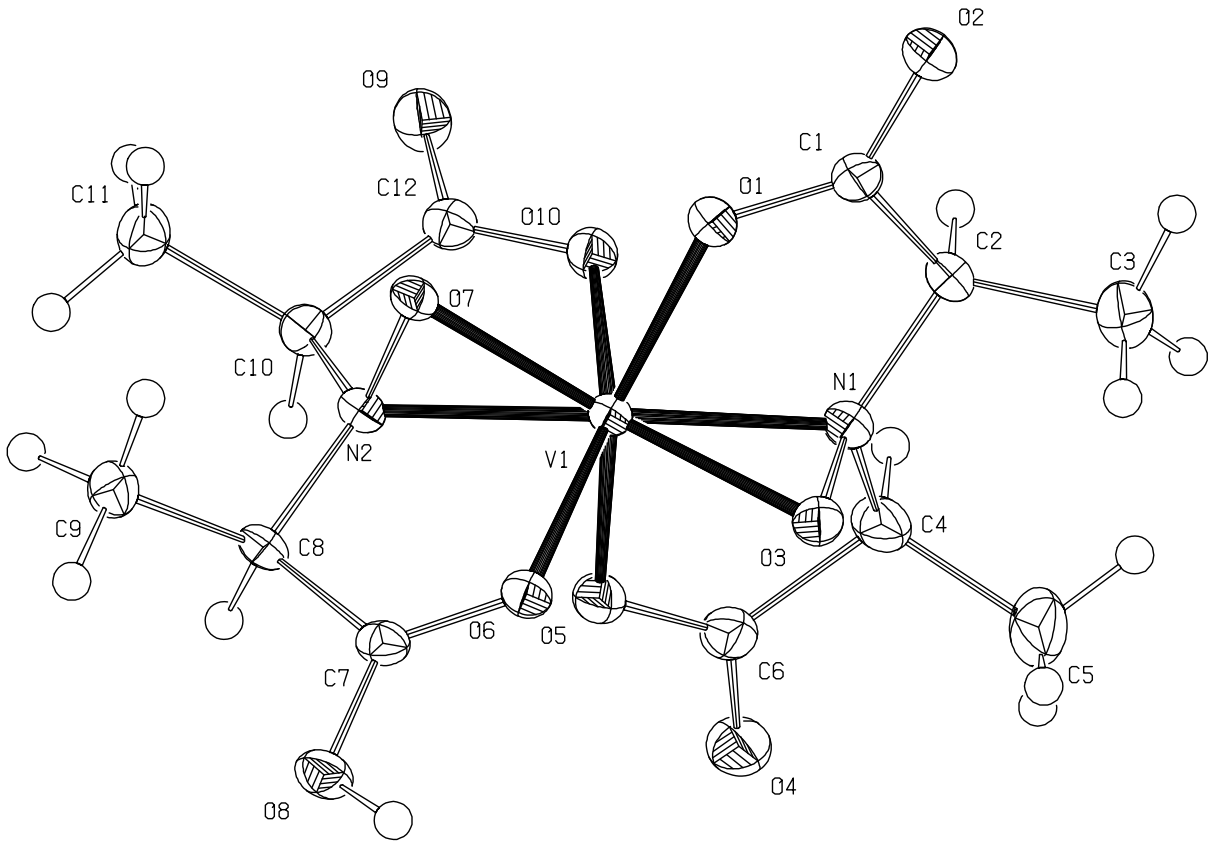


Figure 4.6. *ORTEP view of the 'meso-amavadin' anion. Displacement ellipsoids are drawn at the 50% probability level. The hydronium cation and water molecules are not shown. Selected distances Å: V1-O3 = 1.9599(12), V1-O7=1.9675(12), V1-N1=2.0220(16), V1-N2=2.0337(15),V1-O5=2.0304(13),V1-O10=2.0252(13),V1-O1=2.0662(13),V1-O6=2.0846(13).*

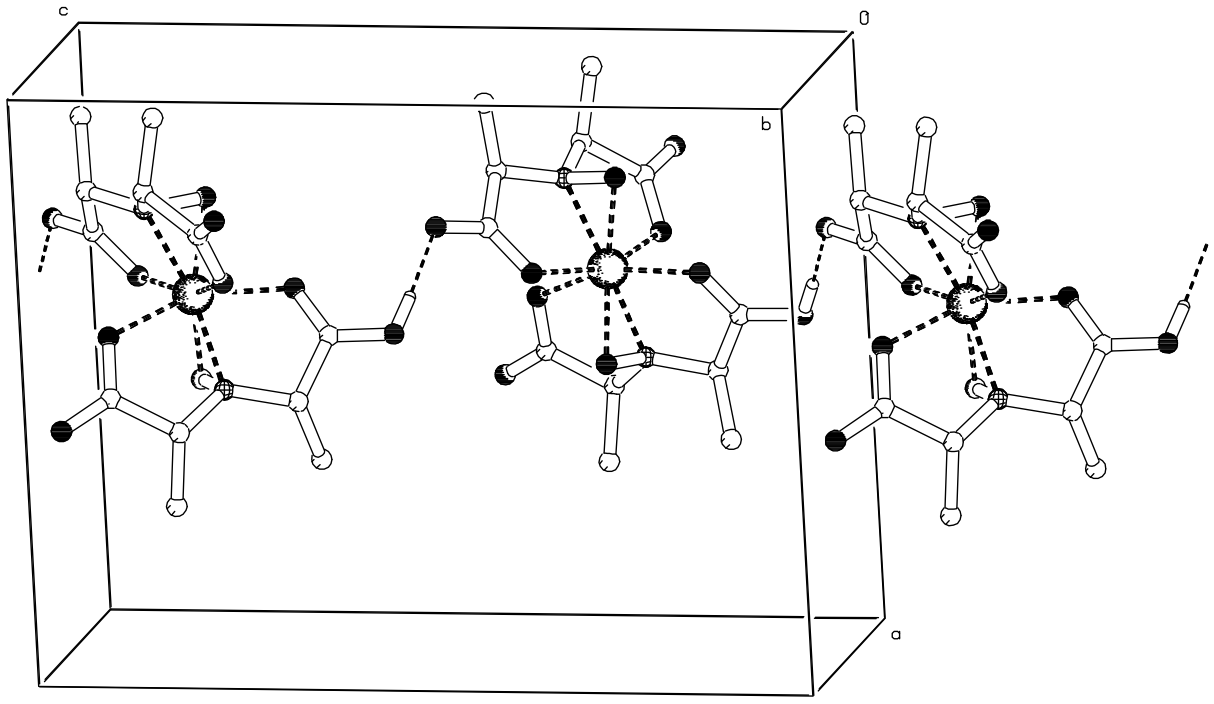
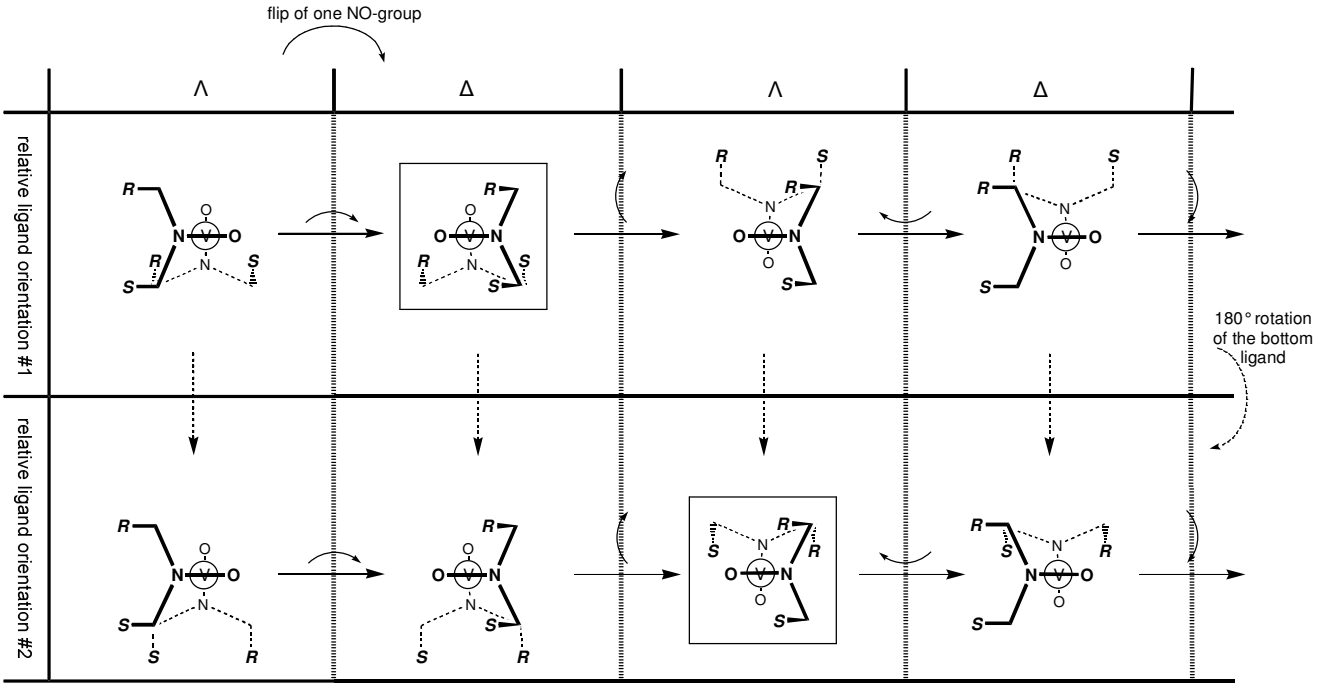


Figure 4.7. *Infinite hydrogen bonded chain of the 'meso-amavadin' anion and its mirror image. Hydrogen bonding with the hydronium cation and water molecules is not shown.*

4.2.3 Proposed model to account for the isomerism and symmetry in amavadin-type complexes

The representations of the structure of natural amavadin as shown in Figure 4.3 in section 4.1.3. clearly account for the presence of two isomeric complexes. In '*meso*-amavadin', however, the situation appeared to be more complicated because the presence of (*R*) and (*S*) stereogenic centers in the ligands breaks the symmetry of the molecule. This results in eight different possibilities to arrange the two *meso*-ligands around the vanadium, instead of the two possibilities for the (*S*,*S*)-ligands. In Scheme 4.3 these are shown schematically from the axial point of view. The configurations of the four backbone carbons are given by the letters ***R*** and ***S*** (located at the respective methyl groups) and the carboxylates are omitted for clarity. It can be seen that the two different relative ligand orientations are not reached by flipping of the N-O moieties, but that they require a 180° rotation of the entire ligand around the N-O bond.



Scheme 4.3. *There are eight possibilities to arrange the two meso-ligands around the vanadium (wedged bonds indicate axial methyl groups, non-wegded bonds indicate equatorial methyl groups).*

In the crystal structure of '*meso*-amavadin', all the methyl groups occupy the axial directions (Figure 4.6).[42] There are two structures in Scheme 4.3 that also have the 'all-axial' conformation (highlighted). They are mirror images (enantiomers) that have an axis of C_2-symmetry and they correspond to the two mirrored vanadium complexes in the symmetric unit of the crystal structure. When comparing the ^{1}H NMR spectrum of oxidized amavadin with that of oxidized '*meso*-amavadin' in Figure 4.5, it is self-evident that the first consists of two diastereomers and the latter of two enantiomers. The absence of other signals in the NMR of oxidized '*meso*-amavadin' demonstrated that a C_2-symmetrical structure is present in solution.

In addition to the two highlighted 'all-axial' conformations in Scheme 4.3, there are two other conformations with C_2-symmetry (shown in the lower left and upper right corners of Scheme 4.3). In contrast, these have an 'all-equatorial' conformation. They can, therefore, be excluded because all the methyl substituents would then occupy the less-favourable positions close to the carboxylates of the other ligand. There is consequently a great certainty that the solution structure is identical with the crystal structure.

Scheme 4.3 shows that the existence of two different relative orientations contributes to the constitution of the eight different ligand arrangements around the vanadium. A change in this relative orientation would require an (imaginary) 180° rotation of the entire ligand around the N-O bond, whereby the two carboxylates change their positions in the equatorial VO_4-plane. This involves their de- and reconnection, a process that is not likely to occur in view of the high stability constants of the complex.[5] The constitution of the two different ligand orientations most likely finds its origin in the ligand complexation during the synthesis of the complex, and can therefore be interpreted as the existence of two modes in which a second ligand can connect to an (imaginary) intermediate mono-ligand complex. This reveals a fundamental difference in how the two Δ and Λ isomers of amavadin and '*meso*-amavadin' are constituted. Moreover, this also implies that the configuration of the vanadium in '*meso*-amavadin' is non-labile, and that – in principle – the isolation of either one of its enantiomeric forms is possible. The fundamental reason for this is that the (*S*,*S*)-ligand is C_2-symmetric, while the *meso*-ligand is not (Figure 4.8). The imaginary 180° rotation would result in an identical structure in case of the (*S*,*S*)-ligand, and in a different structure in the case of the *meso*-ligand. This explanation reveals generality, because the C_2-symmetry of the ligand can also be broken when it carries two different backbone substituents. This results in a model that covers the main aspects of the isomerism in vanadium complexes with amavadin-type ligands. In addition, a solid interpretation of the NMR-data of such complexes should be possible because the symmetry is also accounted for in the model. To test this hypothesis, two amavadin analogues were prepared from ligands that contain two different backbone substituents.

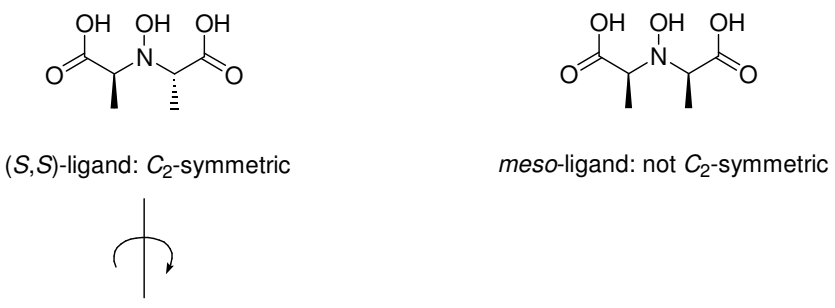
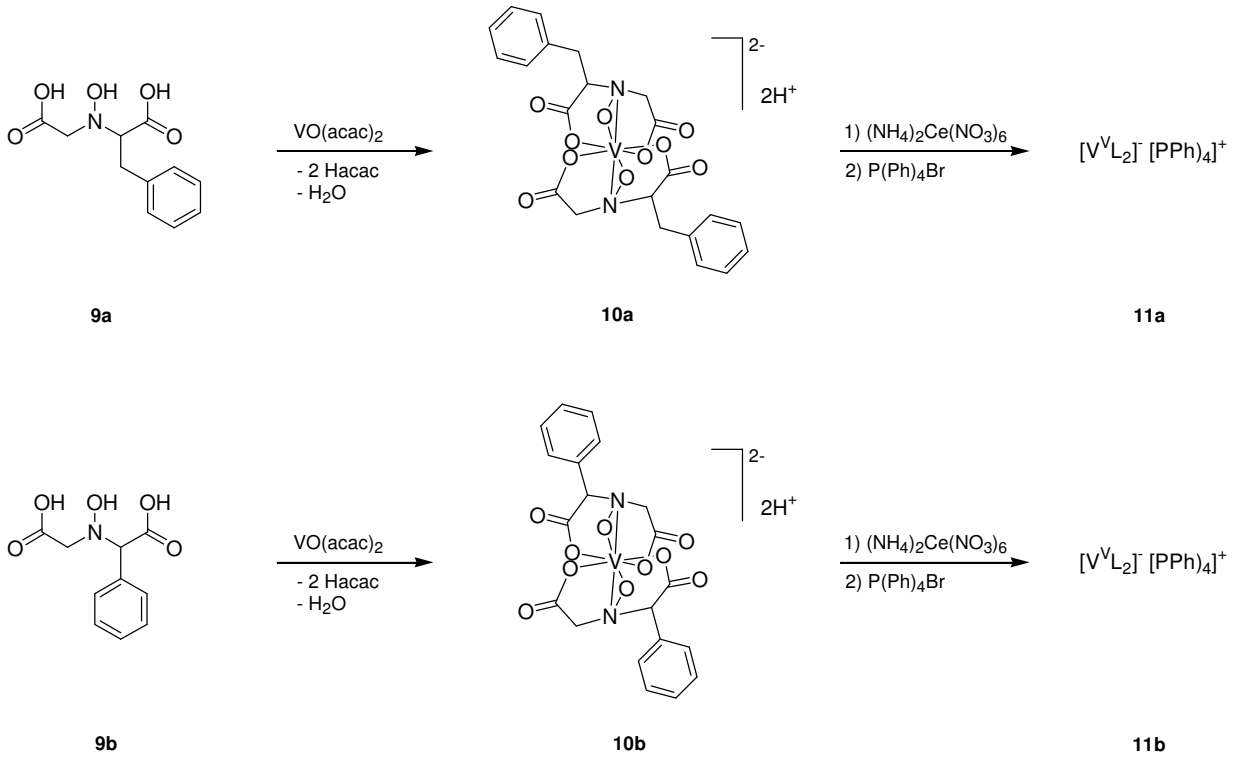


Figure 4.8. *The (S,S)-ligand is C_2-symmetric, but the meso-ligand is not.*

4.3. Synthesis and characterization of amavadin analogues

Two analogues **9a** and **9b** of the natural amavadin ligand were synthesized, which differ in the groups attached to the backbone (Scheme 4.4). Compound **9a** has a backbone hydrogen atom of **2** substituted by a benzyl group, and is therefore chiral. A slightly enantiomerically enriched **9a** was employed for the complexation of vanadium. Compound **9b** has one of the backbone methyl groups of **1** substituted for a phenyl group. It is also chiral and was applied in diastereomerically pure form (racemic). In fact, **9a** and **9b** are constitutional isomers. The aim was to prepare their corresponding vanadium(IV) and (V) complexes **10** and **11** and to study these complexes using NMR in analogy to the studies with **1**. In this way, the model that we proposed in section 4.2.3. can be tested.

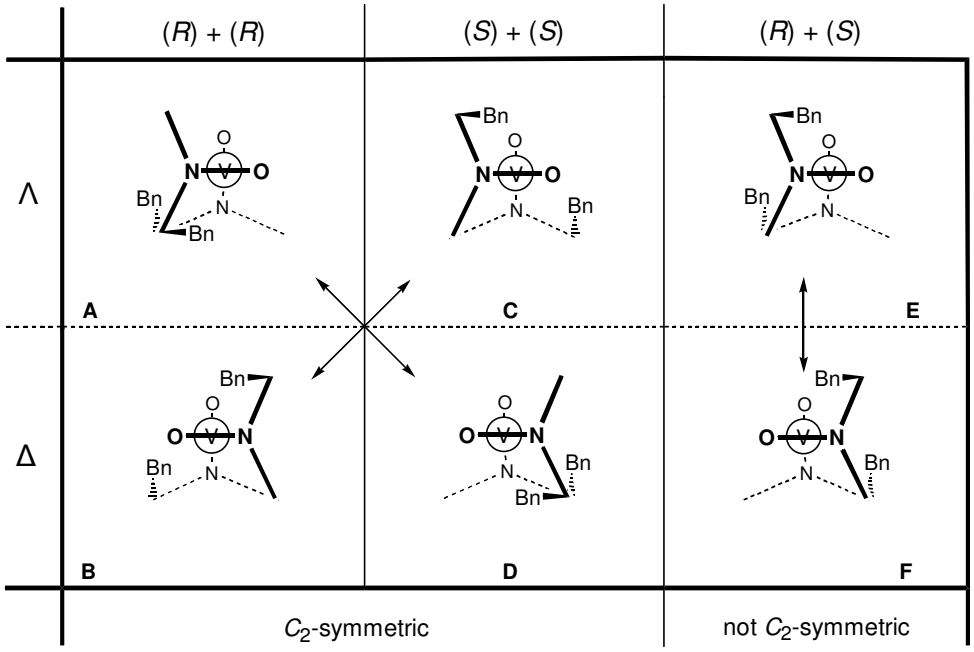


Scheme 4.4. *Formation of the vanadium complexes **10** and **11** from ligands **9**.*

The vanadium(IV) complexes **10** were prepared analogously to the synthesis of amavadin.[29] The increased hydrophobicity of ligands **9** relative to the natural ligand **1** allowed their use in an excess of 5–10%, since they could be washed out with dichloromethane after the complexation. High resolution electrospray mass spectrometry of the new complexes confirmed their identity as **10**. We anticipated that large R-groups would improve the solubility of these complexes in organic solvents, but this appeared not to be the case. The characterization with NMR was performed in analogy to the studies with **1**:[29] the complexes

10 were oxidized in water using $(NH_4)_2Ce(NO_3)_6$ and the resulting vanadium(V) species were extracted into dichloromethane with PPh_4Br to yield the $[VL_2]^-[PPh_4]^+$ complexes **11**.

NMR-studies of 11a. We used the model to predict the appearance of **11a** in the NMR, which is described below. Due to the chiral center in the ligand, there are three combinations with vanadium: 1) two (*R*)-ligands, 2) two (*S*)-ligands and 3) one (*R*)-and one (*S*)-ligand. In analogy to Scheme 4.3, two of the eight possible ligand arrangements of each combination have the 'all-axial' conformation, which results in the presence of **11a** as six isomers: **A**–**F** (Figure 4.9). These isomers consist of the enantiomeric pairs **A+D**, **B+C** and **E+F**, which are indicated by the double-headed arrows. The representations in Figure 4.9 also demonstrate that **A**–**D** have an axis of C_2-symmetry, while **E** and **F** are non-C_2-symmetric species.



*relative abundances of the isomers of **11a**:*	
If (*R*) : (*S*) = x : y, then	(*R*)+(*R*) : (*S*)+(*S*) : (*R*)+(*S*) = x^2 : y^2 : 2xy

Figure 4.9. *Amavadin analogue **11a** is present as six isomers **A**–**F**. The three enantiomeric pairs are indicated by the arrows, their relative abundances are given below.*

In the NMR, the four C_2-symmetric species **A**–**D** are expected to account for half of the signals, while the two non-C_2-symmetric species **E** and **F** account for the other half. Because of the use of a slightly enantiomerically enriched **9a**, half of the signals of **11a** will have a lower integral. The ratio between these integrals is related to the enantiomeric ratio of the ligand as shown in Figure 4.9.

Figure 4.10 shows the ^{1}H NMR of **11a**. The signals of the phenyl group are well separated from the aromatic protons of the PPh_4-counterion (not shown), while the other signals show moderate overlap. It can clearly be seen that the proton in the $NC\underline{H}CH_2$ fragment couples with the two benzylic protons, resulting in a double doublet with coupling constants of J = 2.4 and J = 11.4 Hz. These values can be expected in rigid systems such as **11a**. All protons are represented by four resonances each, which can most clearly be seen for the $NC\underline{H}CH_2$ protons that are represented by four double doublets. This is also the case in the ^{13}C NMR, which shows most resonances grouped per two signals that are very close to each other, or as one signal of higher intensity: there are 2+1+2+2 signals for the carboxylate carbons, 2+2 signals for the two methenyl backbone carbons, 2+2 signals for the two methylene backbone carbons and 2+2 signals for the two benzylic carbons. Furthermore, in both the ^{1}H and ^{13}C NMR all signals are present in an approximate 3 to 4 ratio of signal intensity.

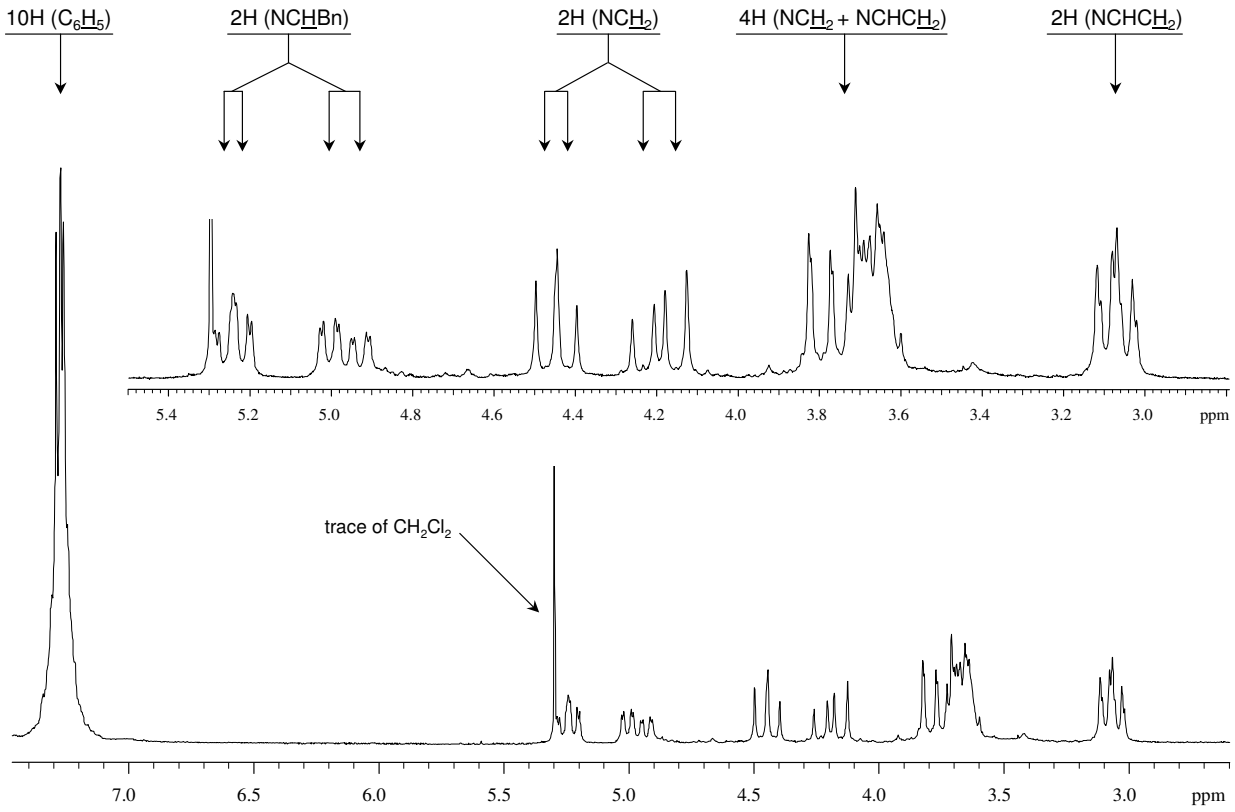


Figure 4.10. *The ^{1}H NMR of complex **11a**.*

The proposed presence of the three diastereomers is confirmed by the NMR data of **11a** and the observed 3 to 4 integral ratio confirms the slight enantiomeric enrichment of the ligand. As in amavadin, it was observed that **11a**, although it is a mixture of diastereomers, gives only one signal in the ^{51}V NMR (–230 ppm).

NMR-studies of 11b. The prediction of the NMR data of **11b** with the model is similar to that of **11a**, because ligands **9** and **10** are both a mixture of enantiomers and both are not C_2-symmetric. The most important difference is that **10** has two substituents on the backbone and **9** only one. If the two stereogenic centra of **10** have opposite configurations, then the ligands will always have their two substituents in the same direction when complexed. By flipping of the N-O moieties, the all-axial situation can be reached (as in '*meso*-amavadin'). If the two stereogenic centra of **10** have the same configuration, then the ligands will always have their two substituents in different directions when complexed. As a result, the complex will always have two axial and two equatorial substituents (as in natural amavadin). Depending on the differences in steric bulk of the methyl and the phenyl substituents, more stereoisomers of **11b** can be formed with such a ligand. Thus, the (*R*,*S*)-(*S*,*R*) as well as the (*R*,*R*)-(*S*,*S*) ligand diastereomer should result in complex **11b** that exists as three pairs of enantiomers. Possibly, more stereoisomers of **11b** exist in the case of the (*R*,*R*)-(*S*,*S*) diastereomer.

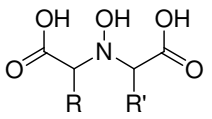
The ^{1}H NMR of oxidized **11b** shows the backbone benzylic protons as four singlets, the backbone methine protons as four quartets and the methyl groups as four doublets, all sharing equal integrals per proton. The signals of the phenyl groups are well separated from the aromatic protons of the PPh_4-counterion. The ^{13}C NMR also shows the carbon signals as two signals very close to each other, or as one signal of higher intensity: e.g. there are 2+1+2+1 carboxylate carbon signals, 2+2 benzylic carbon signals, 2+2 methine carbon signals and 1 methyl carbon resonance. The observation that the signals in the ^{1}H and ^{13}C NMR appear four-fould and in equal intensities demonstrates that the number of isomers of **11b** is equal to the minimum amount that was predicted: half of the molecules **11b** exist as two pairs of C_2-symmetric enantiomers and the other half exist as one pair of non-C_2-symmetric enantiomers (i.e. three diastereomers of **11b** in a ratio of 1:1:2). The ^{51}V NMR of **11b** shows one signal at –243 ppm.

Conclusion: We demonstrated that the model can be used to predict the ^{1}H and ^{13}C NMR spectra of two amavadin analogues that contain a benzyl group or a phenyl group at the ligand backbone.

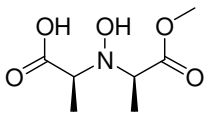
4.4. Complexation of vanadium by *N*-substituted *N*-hydroxy amino acids

It has previously been shown that the modified ligands **2** and **5** do not affect the basic structure of amavadin,[22,24] but that the *N*-hydroxy group is essential for the formation of the non-oxo vanadium(IV) nucleus.[5] We were, therefore, interested in establishing which other factors are important (see introduction of Chapter 3). For this purpose, we synthesized ligands **9**, **12** and **13** (Figure 4.11), in which one backbone methyl group of **1** is replaced by a phenyl or a benzyl

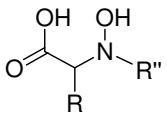
group (**9**); or in which one coordinating group of **1** is replaced by a weaker coordinating center (**12**) or by a non-coordinating alkyl group (**13**).



9a: R = Bn R' = H
9b: R = Ph R' = Me



rac-**12**

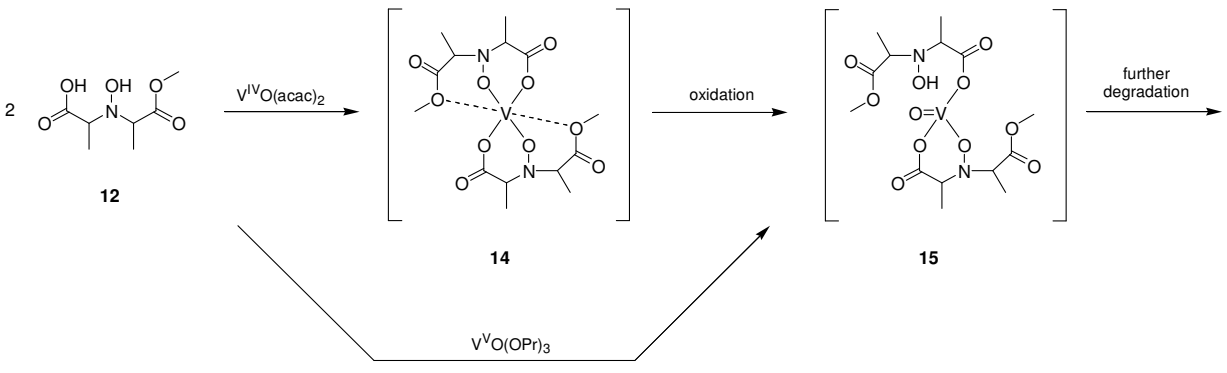


13a: R = Me R' = Ph
13b: R = H R' = Ph
13c: R = Me R' = Et

Figure 4.11. *N-substituted N-hydroxy amino acids available for the complexation of vanadium.*

We showed in section 4.3. that ligands **9** produce the corresponding amavadin analogues **10** (Scheme 4.4). Both complexes were stable in the air and no oxidation occurred. This demonstrates that ligands **9** behave similar to **1**, **2** and **5** and that the presence of larger backbone substituents still leads to an air-stable non-oxo vanadium(IV) nucleus. The complexation of vanadium with **12** and **13** under the same conditions is discussed below.

Compound **12** has one of the carboxylates of ligand **1** protected as the methyl ester; it is thus ideal to establish whether all hydroxy groups in **1** need to be deprotonated in order to complex vanadium(IV) as a non-oxo complex. The complexation was performed by mixing **12** and $VO(acac)_2$ in a 2 : 1 molar ratio in methanol, after which a brown solid was isolated. The molecular mass (HRMS-ESI) corresponded to a vanadium non-oxo complex with two ligands **12**. This can tentatively be assigned to a formal dimethyl ester of amavadin[43] with a hexacoordinated vanadium such as **14** (Scheme 4.5).

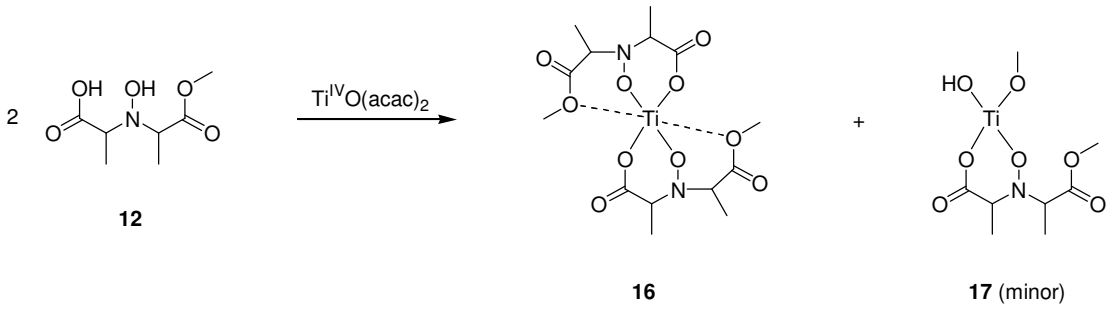


Scheme 4.5. *Schematic representation of the tentative V(IV) and V(V) complexes that are formed from ligand **12**.*

No significant changes were seen in the MS when larger ligand to vanadium ratios were applied. The transient intermediate **14** is oxidized to a complex **15**,[44] which can also be

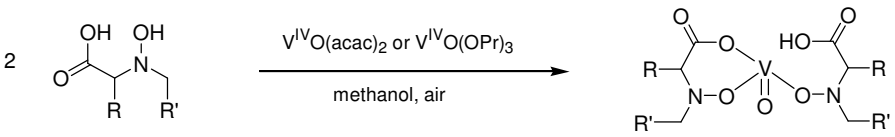
obtained directly by mixing the V(V) precursor $VO(OPr)_3$ with **12**. However, **15** was not stable either; it slowly degraded and was therefore not fully characterized. ^{51}V NMR of the unstable product gave a large resonance at –651 ppm, accompanied by smaller resonances at –605, –623 and –704 ppm. Similarly, the ^{1}H and ^{13}C NMR spectra showed several species and did not allow the exact identification of the complexes. Obviously, **12** does not form an amavadin-like complex with vanadium(IV).

The use of titanium(IV) has proven to be a good substitute for the use of vanadium(IV) to study the complexation in amavadin.[14,15] The advantage of this substitution is that the diamagnetic titanium(IV) nucleus can be measured with NMR.[45] Reaction of **12** with $TiO(acac)_2$ yielded an amorphous yellow solid and the dominant signals in the HRMS (ESI) corresponded to the isotope pattern of bis-ligand titanium complex **16** (Scheme 4.6). The only other signals in the HRMS showed the minor presence of a mono-ligand titanium complex, which can tentatively be assigned to a structure such as **17**. In contrast to the complexation of vanadium, the solution with titanium remained unchanged after exposure to air. Obviously, degradation is limited because the titanium is already in its highest oxidation state. The signals in ^{1}H and ^{13}C NMR of **16** were in accordance with the HRMS data: the main signals represented four inequivalent propionic groups (ester or acid) and two inequivalent methoxy groups, while there was only one set of minor signals.



Scheme 4.6. *Schematic representation of the tentative Ti(IV) complexes that are formed from ligand **12**.*

The complexation of **13a**, **13b** and **13c** with V(IV) and V(V) nuclei was performed by mixing the ligand with either $VO(acac)_2$ or $VO(OPr)_3$ in a 2.1 : 1 molar ratio in methanol. The complexations using $VO(acac)_2$ resulted in brown solutions, that immediately turned green-yellow upon contact with air. The complexations using $VO(OPr)_3$ led to air-stable yellow solutions. The HRMS (ESI) results were similar for either $VO(acac)_2$ or $VO(OPr)_3$: the molecular masses corresponded to those of the oxovanadium(V) complexes **18a**, **18b** and **18c**, which all contain two ligands **13** and one oxo ligand per vanadium[44] (Scheme 4.7). No significant changes were seen in the MS when larger ligand to vanadium ratios were applied. These results demonstrate that ligands **13** are not able to stabilize the V(IV) nucleus.



13a: R = Me R' = Ph
13b: R = H R' = Ph
13c: R = Me R' = Me

18a: R = Me R' = Ph
18b: R = H R' = Ph
18c: R = Me R' = Me

Scheme 4.7. *Schematic representation of the tentative oxovanadium(V) complexes from ligands **13**.*

4.5. Conclusions

Different amavadin stereoisomers and mixtures thereof were prepared and analyzed using NMR. It was observed that the configurations of the backbone carbons have a significant influence on the chemical shifts in the ^{1}H and ^{13}C as well as in the ^{51}V NMR spectra. By using the *meso*-ligand of amavadin, we performed the synthesis and X-ray crystallographic study of '*meso*-amavadin', which revealed to be anionic in the crystal with a hydronium cluster as counter ion. Based on NMR- and X-ray data of amavadin and '*meso*-amavadin', we developed a model that accounts for the structure of amavadin-type complexes, i.e. vanadium(IV) non-oxo complexes that contain two ligands with a tridentate *N*-hydroxyiminodiacetate backbone. The model is based on two properties of the complex: 1) the configurationally labile vanadium and 2) the existence of two different relative ligand orientations. It explains how these properties result in different modes to arrange the two ligands around the vanadium and it accounts for eventual symmetry in the complex. We demonstrated that the model can be used to predict the ^{1}H and ^{13}C NMR spectra of two amavadin analogues that contain a benzyl or a phenyl group at the ligand backbone.

The second aim of this study on the vanadium(IV) non-oxo nucleus in amavadin was to investigate if large ligand backbone substituents can be accomodated for and to which extent the carboxylate groups are essential. The complexation studies showed that increasing the size of the methyl backbone substituents by introducing a benzyl or a phenyl group still gives V(IV) non-oxo complexes that are analogous to amavadin. In contrast, the protection of one carboxylate per ligand does not give a stable vanadium(IV) non-oxo compound. Instead, the initially obtained complex is slowly oxidized in the air. The complete lack of one carboxylic acid group results in immediate oxidation. In conclusion, these results show that, in addition to the secondary *N*-hydroxy group, the two unprotected carboxylates are also necessary to obtain an air-stable vanadium(IV) non-oxo nucleus.

4.6. Experimental

General considerations. ^{1}H, ^{13}C and ^{51}V NMR spectra were recorded on a Varian VXR-400S (400 MHz) or a Varian Unity Inova 300 (300 MHz) instrument. Chemical shifts of the ^{1}H and ^{13}C nuclei are expressed in parts per million (δ) relative to tetramethylsilane (in $CDCl_3$) or *t*-BuOH (in H_2O). Chemical shifts of the ^{51}V nuclei are expressed in parts per million (δ) relative to the external reference $VOCl_3$. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet). Low resolution mass spectroscopy was measured on a Micromass Quattro LC-MS spectrometer (Electron Spray Ionization). High resolution mass spectroscopy was measured on a Thermo LTQ Orbitrap; resolution 50000; mass accuracy <2 ppm; samples were diluted with a 1 : 1 methanol/water solution; injection volume: 10 µl; flowrate: 50 µl/min; eluent is a 1 : 1 methanol/water solution. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Melting points were measured on a Büchi 510 and are uncorrected. $VO(acac)_2$ was recrystallized from acetone[46] prior to use. All other chemicals and dry solvents were obtained from Aldrich or Merck and were used as received. Other solvents were purchased from Baker. Ultrapure water (MilliQ) was used.

Synthesis of the amavadin isomers and their oxidation to $[VL_2]^-[PPh_4]^+$. The synthesis of the amavadin isomers from the ligand samples **1a**–**1d**, their oxidation and their isolation as their PPh_4-salts were performed according to procedures reported in Chapter 2, except that $MgSO_4$ was used as a drying agent instead of $CaCl_2$.

NMR data of $[VL_2]^-[PPh_4]^+$ using ligand sample 1a. [= (*OC*-6-1'3)-tetraphenylphosphonium (bis(di(*S*-1-carboxy-κ*O*-ethyl)-η^2-azanolato)vanadium(V))]. These data were reported in the experimental part of chapter 2.

NMR data of $[VL_2]^-[PPh_4]^+$ using ligand sample 1b. [= (*OC*-6-1'3)-tetraphenylphosphonium (bis((*R*-1-carboxy-κ*O*-ethyl)(*S*-1-carboxy-κ*O*-ethyl)-η^2-azanolato)vanadium(V))]. ^{1}H NMR (300 MHz, $CDCl_3$) δ; 1.66 (d, 6H, $CHC\underline{H}_3$, *J* = 7.3 Hz), 1.68 (d, 6H, $CHC\underline{H}_3$, *J* = 7.3 Hz), 4.80 (q, 2H, $C\underline{H}CH_3$, *J* = 7.3 Hz), 5.06 (q, 2H, $C\underline{H}CH_3$, *J* = 7.3 Hz), 7.65, 7.77, 7.92 (m, 20H, $P(C_6\underline{H}_5)_4$. ^{13}C NMR (75 MHz, $CDCl_3$) δ; 173.39, 172.172 (4C, $\underline{C}OOH$), 135.84 (4C, $P(\underline{C}_6H_5)_4$), 134.39 (8C, $P(\underline{C}_6H_5)_4$), 130.75 (8C, $P(\underline{C}_6H_5)_4$), 117. 46 (d, 4C, P-$\underline{C}$, $^{1}J_{P\text{-}C}$ = 89 Hz), 72.31, 71.55 (4C, $\underline{C}HCH_3$), 19.00 (4C, $CH\underline{C}H_3$). ^{51}V NMR (79 MHz, $CDCl_3$, 25 °C) δ; –257.

NMR data of $[VL_2]^-[PPh_4]^+$ using ligand sample 1c. Ligand sample **1c** is not stereochemically pure, and as a result the ^{1}H NMR and ^{13}C NMR contain too many signals for proper assignments. Only the ^{51}V NMR data are given. ^{51}V NMR (79 MHz, $CDCl_3$, 25 °C) δ; –272, –291 (overlapping).

NMR data of $[VL_2]^-[PPh_4]^+$ using ligand sample 1d. Ligand sample **1d** is not stereochemically pure, and as a result the ^{1}H NMR and ^{13}C NMR contains too many signals for proper assignments. Only the ^{51}V NMR data are given. ^{51}V NMR (79 MHz, $CDCl_3$, 25 °C) δ; –258, –272, –289 (overlapping).

Crystal Structure Determination of '*meso*-amavadin'. A blue crystal was used for data collection on a Nonius KappaCCD diffractometer on rotating anode with MoKα radiation at 150 K. Crystal data: $C_{12}H_{17}N_2O_{10}V.H_5O_2.2(H_2O)$, Monoclinic, $P2_1/c$, a = 11.8487(12) Å, b = 10.7262(12) Å, c = 15.6163 Å, β = 92.682(14)°, V = 1982.5(4) Å^3. A total of 53421 reflections up to Θ = 27.5° were measured and

averaged into a unique set of 4516 reflections (Rint = 0.068). The structure was solved by Direct Methods (SHELXS86[47]) and refined with SHELXL97[48] to R = 0.0346 for 3580 reflections with I > 2 σ (I), wR2 = 0.0858, S = 1.02, residual density excursions between –0.33 and 0.34 e/Å^3. H-atoms on carbon were introduced at calculated positions and refined riding on their carrier atoms. Hydrogen atoms on oxygen were identified from difference Fourier maps and their positions refined. An $[O_2H_5]^+$ cation was positively identified in a contoured map. Another contoured map involving the short H-bond between O2 and O8 indicated a disordered H-atom position with the major component near O8. No attempt was made to refine a disorder model. Illustrations and structure validation were done with PLATON.[49] CCDC 624773 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html.

Synthesis of 10a [= (*OC*-6-1'3)-dihydrogen(bis((carboxy-κ*O*-methyl)(1-carboxy-κ*O*-2-phenyl-ethyl)-η^2-azanolato)vanadium(IV))]. To a solution of 40 mg (0.168 mmol) of **9a** in 15 mL of ethanol was added 21 mg (0.080 mmol) of $VO(acac)_2$. The mixture immediately coloured deep brown for a few seconds. After stirring for 20 minutes, the mixture was clear and blue-purple and the smell of Hacac was noticed. All volatiles were removed by rotary evaporation and the residue was stripped twice with methanol. The dry residue was then washed twice with dichloromethane, yielding 41 mg (0.078 mmol, 98%) of **10a** as a glittering blue solid. Mp = 130 °C (decomp.). $[\alpha]_D^{20}$ = –6.0° (c=0.4, H_2O). HRMS-ESI calcd. for $C_{22}H_{23}N_2O_{10}$ $[M+H]^+$ 526.0787, found 526.0788. HRMS-ESI calcd. for $C_{22}H_{21}N_2O_{10}$ $[M–H]^–$ 524.0641, found 524.0639.

Oxidation of 10a to 11a. Complex **10a** was oxidized and isolated using the procedure reported in Chapter 2, except that $MgSO_4$ was used as a drying agent instead of $CaCl_2$. Due to the non-racemic ligand, diastereoisomers are formed in a ratio of A : B = 3 : 4. The assignment of the ^{1}H and ^{13}C resonances was based on this ratio. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C) δ; 3.02–3.12 (m, 4H, $C\underline{H}_2C_6H_5^A$ + $C\underline{H}_2C_6H_5^B$), 3.60–3.83 (m, 8H, $C\underline{H}_2C_6H_5^A$ + $C\underline{H}_2C_6H_5^B$ + $NC\underline{H}_2^A$ + $NC\underline{H}_2^B$), 4.15 (d, 1H, $NC\underline{H}_2^B$, *J* = 15.9 Hz), 4.23 (d, 1H, $NC\underline{H}_2^A$, *J* = 16.2 Hz), 4.43 (d, 1H, $NC\underline{H}_2^A$, *J* = 15.9 Hz), 4.48 (d, 1H, $NC\underline{H}_2^B$, *J* = 16.2 Hz), 4.93 (dd, 1H, $NC\underline{H}^A$, *J* = 2.4 Hz and *J* = 11.4 Hz), 5.01 (dd, 1H, $NC\underline{H}^B$, *J* = 2.4 Hz and *J* = 11.4 Hz), 5.23 (dd, 1H, $NC\underline{H}^B$, *J* = 2.4 Hz and *J* = 11.4 Hz), 5.27 (dd, 1H, $NC\underline{H}^A$, *J* = 2.4 Hz and *J* = 11.4 Hz), 7.22–7.35 (m, 20H, $C_6\underline{H}_5^{A+B}$), 7.65, 7.77, 7.92 (m, 40H, $P(C_6\underline{H}_5)_4^{A+B}$. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 172.11^B, 172.09^A, 171.18^{A+B}, 171.01^A, 170.96^B, 169.93^B, 169.89^A (8C, $\underline{C}OOH$), 137.88^A, 137.86^B, 137.57^B, 137.55^A, 129.45^B, 129.42^A, 129.04^A, 129.00^{B+B}, 128.94^A, 127.81^A, 127.78^B (24C, $\underline{C}_6H_5$), 136.12 (8C, $P(\underline{C}_6H_5)_4^{A+B}$), 134.65 (16C, $P(\underline{C}_6H_5)_4^{A+B}$), 130.97 (16C, $P(\underline{C}_6H_5)_4^{A+B}$), 117.72 (d, 8C, P-$\underline{C}^{A+B}$, $^1J_{P\text{-}C}$ = 89 Hz), 78.58^B, 78.46^A, 78.00^A, 77.93^B (4C, $N\underline{C}HC_6H_5$), 66.42^A, 66.32^B, 65.54^{A+B} (4C, $N\underline{C}H_2$), 39.57^A, 39.54^B, 39.51^B, 39.49^A (4C, $\underline{C}H_2C_6H_5$). ^{51}V NMR (79 MHz, $CDCl_3$, 25 °C) δ; –230.

Synthesis of 10b [= (*OC*-6-1'3)-dihydrogen((bis(1-carboxy-κ*O*-ethyl)(1-carboxy-κ*O*-1-phenyl-methyl)-η^2-azanolato)vanadium(IV))]. To a solution of 44 mg (0.184 mmol) of **9b** in 15 mL of ethanol was added 22 mg (0.084 mmol) of $VO(acac)_2$. The mixture immediately coloured deep brown

for a few seconds. After stirring for 20 minutes, the mixture was clear and blue-purple and the smell of Hacac was noticed. All volatiles were removed by rotary evaporation and the residue was stripped twice with methanol. The dry residue was then washed twice with dichloromethane, yielding 42 mg (0.080 mmol, 96%) of **10b** as a glittering blue solid. Mp = 200 °C (decomp.). HRMS-ESI calcd. for $C_{22}H_{23}N_2O_{10}$ $[M+H]^+$ 526.0787, found 526.0786. HRMS-ESI calcd. for $C_{22}H_{21}N_2O_{10}$ $[M–H]^-$ 524.0641, found 524.0636.

Oxidation of 10b to 11b. Complex **10b** was oxidized and isolated using the procedure reported in Chapter 2, except that $MgSO_4$ was used as a drying agent instead of $CaCl_2$. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C) δ; 0.946 (4 x d (overlapping), 6H, CHC<u>H</u>$_3$), 4.88, 4.89, 5.11, 5.13 (4 x q, 2H, C<u>H</u>CH$_3$), 5.82, 5.83, 6.07, 6.10 (4 x s, 2H, C<u>H</u>C$_6$H$_5$), 7.30–7.46 (m, 10H, C$_6$<u>H</u>$_5$), 7.65, 7.77, 7.92 (m, 20H, P(C$_6$<u>H</u>$_5$)$_4$$^{A+B}$. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ 174.09, 173.97, 172.81, 171.98, 171.91, 170.72 (4C, <u>C</u>OOH), 136.95, 136.80, 136.76, 129.86, 129.70, 129.03 (12C, <u>C</u>$_6$H$_5$), 80.63, 80.49, 79.70, 79.51 (2C, <u>C</u>HC$_6$H$_5$), 72.96, 72.80, 72.38, 72.17 (2C, <u>C</u>HCH$_3$), 18.87 (2C, CH<u>C</u>H$_3$). ^{51}V NMR (79 MHz, $CDCl_3$, 25 °C) δ; –243.

Synthesis of 14. To a suspension 38 mg (0.200 mmol) of **12** in 15 mL of dry dichloromethane under a nitrogen atmosphere were added 27 mg (0.100 mmol) of $VO(acac)_2$. After stirring for four hours, the solution was a brown and the smell of Hacac was noticed. All volatiles were removed by rotary evaporation and the residue was stripped twice with dry dichloromethane, yielding 42 mg (0.098 mmol, 98%) of **14** as a glittering brown solid. Mp = 130 °C (decomp.).

Complexation of ligands 12 and 13 to $VO(acac)_2$: solutions for HR-MS measurements. To a solution of 0.021 mmol of the ligand in 0.8 mL of methanol were added 0.010 mmol of $VO(acac)_2$. The mixture was stirred in an open flask for one hour. A grey-purple solution was obtained with **14**, while **15** and **19** gave pale yellow solutions. These solutions were measured with electrospray HRMS (Table 4.2 and Table 4.3).

Complexation of ligands 12 and 13 to $VO(OPr)_3$: solutions for HR-MS measurements. To a solution of 0.074 mmol of the ligand in 2 mL of methanol were added 0.035 mmol of $VO(OPr)_3$. The mixture was stirred in an open flask for one hour, after which yellow solutions were obtained. These solutions were measured with electrospray HRMS (Table 4.2 and Table 4.3).

Table 4.2. *HRMS-ESI of tentative complexes **14** and **15** using ligand **12** (=L).*

Product	Molecule ion			$VO(acac)_2$ + 2L	$VO(OPr)_3$ + 2L
	ionization	bruto formula	calculated MM	found MM	found MM
14	$[M+H]^+$	$C_{14}H_{23}N_2O_{10}V$	430.0787	430.0786	n.a.
	$[M–H]^-$	$C_{14}H_{21}N_2O_{10}V$	428.0641	–	
15	$[M+H]^+$	$C_{13}H_{20}N_2O_{10}V$	415.0553	415.0551	415.0551
	$[M–H]^-$	$C_{13}H_{18}N_2O_{10}V$	413.0406	413.0401	413.0400

Table 4.3. *HRMS-ESI of tentative complexes **18a**, **18b** and **18c** using ligands **13a**, **13b** and **13c** (=L).*

Product	Molecule ion			$VO(acac)_2$ + 2L	$VO(OPr)_3$ + 2L
	ionization	bruto formula	calculated MM	found MM	found MM
18a	$[M+H]^+$	$C_{20}H_{24}N_2O_7V$	455.1018	455.1015	455.1016
	$[M-H]^-$	$C_{20}H_{22}N_2O_7V$	453.0872	453.0865	453.0865
18b	$[M+H]^+$	$C_{18}H_{20}N_2O_7V$	427.0705	427.0705	427.0704
	$[M-H]^-$	$C_{18}H_{18}N_2O_7V$	425.0559	425.0552	425.0553
18c	$[M+H]^+$	$C_{10}H_{20}N_2O_7V$	331.0705	331.0703	331.0703
	$[M-H]^-$	$C_{10}H_{18}N_2O_7V$	329.0559	329.0554	329.0554

Synthesis of 16. To a suspension 19 mg (0.100 mmol) of **12** in 15 mL of dry dichloromethane under a nitrogen atmosphere were added 13 mg (0.049 mmol) of $TiO(acac)_2$. After stirring for four hours, the reaction mixture had become a light yellow suspension, and the smell of Hacac was noticed. All volatiles were removed by rotary evaporation and the residue was stripped twice with dry dichloromethane. The yellow powder was suspended in dichloromethane and the suspension was centrifuged. Evaporation of the supernatant yielded 20 mg (0.047 mmol, 97%) of **16** as a fine yellow powder. Mp = 130 °C (decomp.). ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42, 1.43, 1.67, 1.74 (4 x d, 12H, CHC$\underline{H}_3$), 3.75 (s, 6H, OC$\underline{H}_3$), 3.84, 3.87 (2 x q, 2H, C$\underline{H}$CH$_3$), 4.06 (s, 6H, OC$\underline{H}_3$), 4.36, 4.75 (2 x q, 2H, C$\underline{H}$CH$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 180.41, 175.61, 174.34, 172.32 (4C, $\underline{C}$OOH), 68.11, 65.59, 62.39, 61.41, 56.45, 52.16 (6C, 4 x $\underline{C}$H CH$_3$ + 2 x O$\underline{C}$H$_3$), 15.86, 15.32, 14.91, 13.69 (4C, CH$\underline{C}$H$_3$). HRMS-ESI: see Table 4.4.

Table 4.4. *HRMS-ESI of tentative titanium complexes **16** and **17** using ligand **12** (=L).*

Product	Molecule ion			$TiO(acac)_2$ + L	
	ionization	bruto formula	calculated MM	found MM	
16	$[M-H]^-$	$C_{14}H_{21}N_2O_{10}Ti$	425.0676	425.0675	bis-ligand complex
17	$[M-H]^-$	$C_8H_{14}NO_7Ti$	284.0250	284.0253	mono-ligand complex

Synthesis of titanium substituted amavadin.[45] To a suspension of 29.3 mg (0.11 mmol) of $TiO(acac)_2$ in 5 mL of H_2O was added an aqueous solution of 40 mg (0.22 mmol) of ligand **1a**. After stirring for 1 hour, the mixture was almost clear and yellow. All volatiles were removed by rotary evaporation, and the resulting yellow solid was redissolved in water. After centrifugation the solution was clear and the yellow solution was decanted and evaporated to dryness, yielding 45 mg (11 mmol, quantitatively) of the titanium substituted amavadin as a yellow solid. Melting point: 130 °C (decomp.). Two isomers were formed in a ratio of A : B = 8 : 10. The assignment of the ^{1}H and ^{13}C resonances was based on this ratio. ^{1}H NMR (400 MHz, D_2O) δ; 1.39^A, 1.41^B (d, 6H, CHC$\underline{H}_3$, J = 7.2 Hz), 1.51^B, 1.57^A (d, 6H, CHC$\underline{H}_3$, J = 7.2 Hz), 4.05^B, 4.11^A (q, 2H, C$\underline{H}$CH$_3$, J = 7.2 Hz), 4.40^A, 4.53^B (q, 2H, C$\underline{H}$CH$_3$, J = 7.2 Hz). ^{13}C NMR (100 MHz, D_2O) δ; 182.85^B, 182.43^A, 181.48^A, 181.41^B (4C, $\underline{C}$OOH), 68.31^A,

67.76^{B}, 64.71^{B}, 64.02^{A} (4C, $\underline{C}HCH_3$), 15.80^{B}, 15.52^{A}, 13.05^{A+B} (4C, $CH\underline{C}H_3$). MS (*m*/*z*) 399.4 (ES^+, M+1), 797.1 (ES^+, 2M+1), 397.4 (ES^-, M–1), 795.1 (ES^-, 2M–1).

4.7. Acknowledgements

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40. The singlet at 2.17 ppm is due to a trace of acetone.
41. The X-ray determination of natural amavadin (i.e. $[V(S,S\text{-}LH)_2]\cdot 4H_2O$) has been mentioned in the literature (ref. 24), but the authors of ref 24 refer to unpublished work.
42. The reported crystal structure of the vanadium(IV) complex with *meso*-ligand **4** also has the 'all-axial' conformation.[24]
43. It was attempted to prepare **14** by reaction of natural amavadin with diazomethane, but no reaction was observed.
44. The bruto formulae of **15** and **18** require one of the ligand oxygen atoms to be protonated; the exact location of the proton as drawn is speculative.
45. This was once more confirmed by performing the complexation of the naturally occurring (*S*,*S*)-amavadin ligand (**1a**) to titanium, which gave ^{1}H and ^{13}C NMR-spectra almost identical to those obtained from **1a** with vanadium (Figure 4.5).
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5 STUDY OF AMAVADIN AND OXIDIZED AMAVADIN IN SOLUTION

5.1. Introduction

Since its first isolation in 1972,[1] amavadin has been subject of studies that mainly concentrated on its (hydrolytic) stability, its titration, its redox behaviour (cyclic voltammetry) and its paramagnetism (EPR).[2,3,4,5,6] In a few of these and other cases, amavadin was also analyzed by means of IR, NMR and elemental analysis.[7] The reported synthetic procedures for amavadin generally use a mixture of ligand diastereomers that is not free of sodium-salts, and the amavadin is mainly isolated with sodium, calcium or ammonium counterions. The synthesis of chemically and optically pure amavadin (Chapter 2)[8] allowed new studies of its behaviour. This chapter describes investigations of the solution properties of amavadin with the aid of NMR, UV-VIS, IR, Raman and MS. Sections 5.2 and 5.3 exclusively discuss amavadin in its V(IV) oxidation state, while the latter sections also deal with V(V) forms of amavadin and the cycling between the two oxidation states. The latter studies mainly concern the interaction of amavadin with hydrogen peroxide (H_2O_2) and *tert*-butyl hydroperoxide (TBHP) under the conditions that are applied in catalytic oxidations (Chapter 6).

5.2. Interaction of amavadin with water

Amavadin has been isolated by other researchers containing different equivalents of water, and the colours they assigned to solid amavadin ranged from blue to purple. We observed that amavadin both in solution and in the solid state can have different colours, which gave rise to more detailed investigations on the interaction of amavadin with water.

5.2.1. Dehydration of amavadin

When a solution of amavadin is evaporated in the air or under vacuum at room temperature, an amorphous blue solid is obtained. However, after prolonged drying under high vacuum (<1 mbar) and at higher temperatures (>50 °C) the material gradually breaks into a fine purple powder accompanied by weight loss. When the purple powder is dissolved in water and subsequently evaporated until the blue solid is obtained, the product has gained 5–10% of weight. This observed dehydration/rehydration is not reported explicitly in earlier publications and might not seem an extraordinary property, but it can explain the confusion in the literature about the exact composition of amavadin. In all cases where chemically pure amavadin was claimed to be isolated, the elemental analyses corresponded to amavadin that contains a certain amount of water. For a discussion on this subject it is important to realize that the assumed composition of amavadin has changed in time. Initially,[1] the bruto formula of solid amavadin was thought to be $C_{12}H_{20}N_2VO_{11}$ (i.e. VOL_2H_4, a vanadium-oxo compound), while later[9] it was proven to be $C_{12}H_{18}N_2VO_{10}$ (i.e. VL_2H_2, a vanadium *non*-oxo compound). The difference

between these is one equivalent of water. After the first isolation of amavadin in 1972,[1] the elemental analysis of the sample was said to correspond to amavadin·1H_2O, which can now be considered as amavadin·2H_2O. The same bruto formulae were reported by Riechel and Nawi[2] and by Kneifel and Bayer[10] for their chemically synthesized amavadin. After the discovery that amavadin is a non-oxo compound, Garner *et al.*[7] reported that the measured elemental analysis of their isolated amavadin matched amavadin·3H_2O. It was also this group that found that amavadin crystallizes as amavadin·4H_2O. Unfortunately, they did not publish these data but only briefly referred to them as 'unpublished results' in another publication.[4] The crystal structure that we obtained from '*meso*-amavadin' (Chapter 4) also contained four molecules of water per vanadium, which supported the unpublished crystal data. We also performed elemental analysis on our samples of amavadin, but the values we obtained were inconsistent. Support for the presence of amavadin·0H_2O was obtained via a procedure to prepare amavadin in a known molar amount: the vanadium precursor and the ligand were mixed in an exact 1 to 2 ratio in water, followed by lyophilisation (Chapter 2). In all amavadin preparations that we performed in this way (more than 15, some of which on a one gram scale), quantitative yields were obtained when the yield calculation was based on zero equivalents of water (amavadin·1H_2O would already correspond to 104% of the expected mass). The colour of the lyophilisate powder was initially light blue and changed to purple after a few days of storage in a closed flask. When the water was evaporated instead of lyophilized, amavadin·0H_2O could also be obtained as a purple powder in quantitative yield, but only after extensive drying.[11] Furthermore, dry amavadin is hygroscopic: it gains weight when left in the air and a dry blue solid is obtained after one day.

5.2.2. Hydration of amavadin in aqueous solution

Although it was shown that the dehydration and the rehydration of amavadin are fully reversible processes, it was striking to see that dry amavadin dissolved in water under the formation of a red solution, that became purple within a minute and regained its blue colour only after two hours. When the dry amavadin was dissolved in methanol, the mixture remained red. Apparently, the rehydration is accompanied by a certain change in the complex due to the water. This change can for example be isomerization, dimerization or a reaction with water.This process was studied with several spectroscopic techniques. First, it was followed with UV-VIS spectroscopy. Spectra of a freshly prepared 5 mM aqueous solution of dry amavadin were recorded at time intervals of 6 minutes (Figure 5.1). There were significant changes of intensity between the initial and the final absorption spectra while the position of the absorption maximum moved from 537 to 563 nm. There is an isosbestic point at 649 nm, indicating a process in which one new species is formed at the expense of the initial species without the formation of an intermediate.

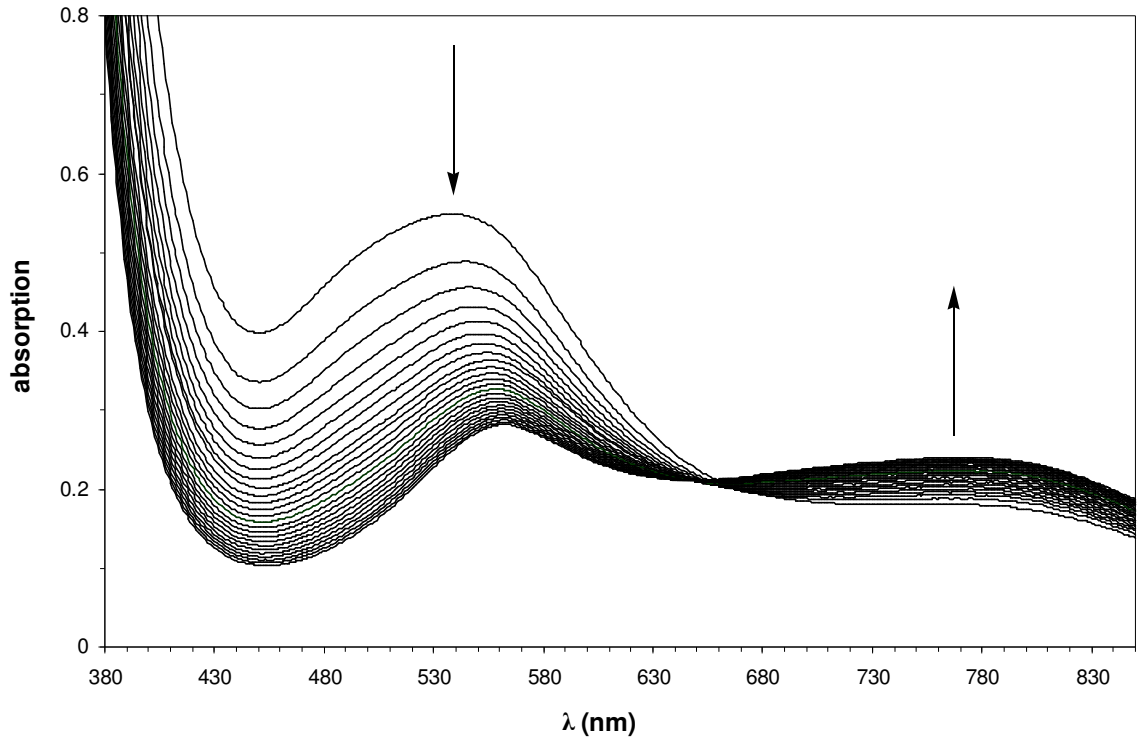


Figure 5.1. *Changes in the UV-VIS during 150 minutes after dissolving dry amavadin in water.*

Next, the process was followed with Raman spectroscopy and with IR spectroscopy. For the latter, a technique called 'attenuated total reflection' was used (ATR), which allowed for *in situ* real time IR measurements.[12] The spectra of both techniques were recorded at regular time intervals, but they showed only minor changes over a period of two hours (Figure 5.2; only the initial and final spectra are shown). The Raman shifts occurred at exactly the same frequencies, while only small differences in intensity were observed throughout the spectrum. The shifts below 700 cm^{-1} are most intense, and typically represent the vibrations of V-O bonds with a bond order of <1.[13] The shifts between 1500 and 700 cm^{-1} are due to ligand vibrations. The IR absorptions at 1597 and 1466 nm have shifted a few cm^{-1} to higher and lower wavenumber, respectively, while a weak absorption has emerged at 1220 nm. The IR-spectrum bears much similarity with the solid state spectrum (Chapter 2), both clearly showing the absorptions at 1600, 1458, 1390, 1128, 1084, and 980 cm^{-1}. The ^{1}H, ^{13}C and ^{51}V NMR recorded after oxidation of the species that were present before and after the 150 minutes of isomerization gave spectra that were identical to each other and to those obtained previously (Figure 4.5 on p. 94). However, it must be kept in mind that subtle changes in the complex are likely to get lost after oxidation of the vanadium. Finally, electrospray MS measurements of the initial and the final species showed that the molecular mass remained the same during the process: it was 401, the mass of amavadin ($=V^{IV}L_2H_2$).

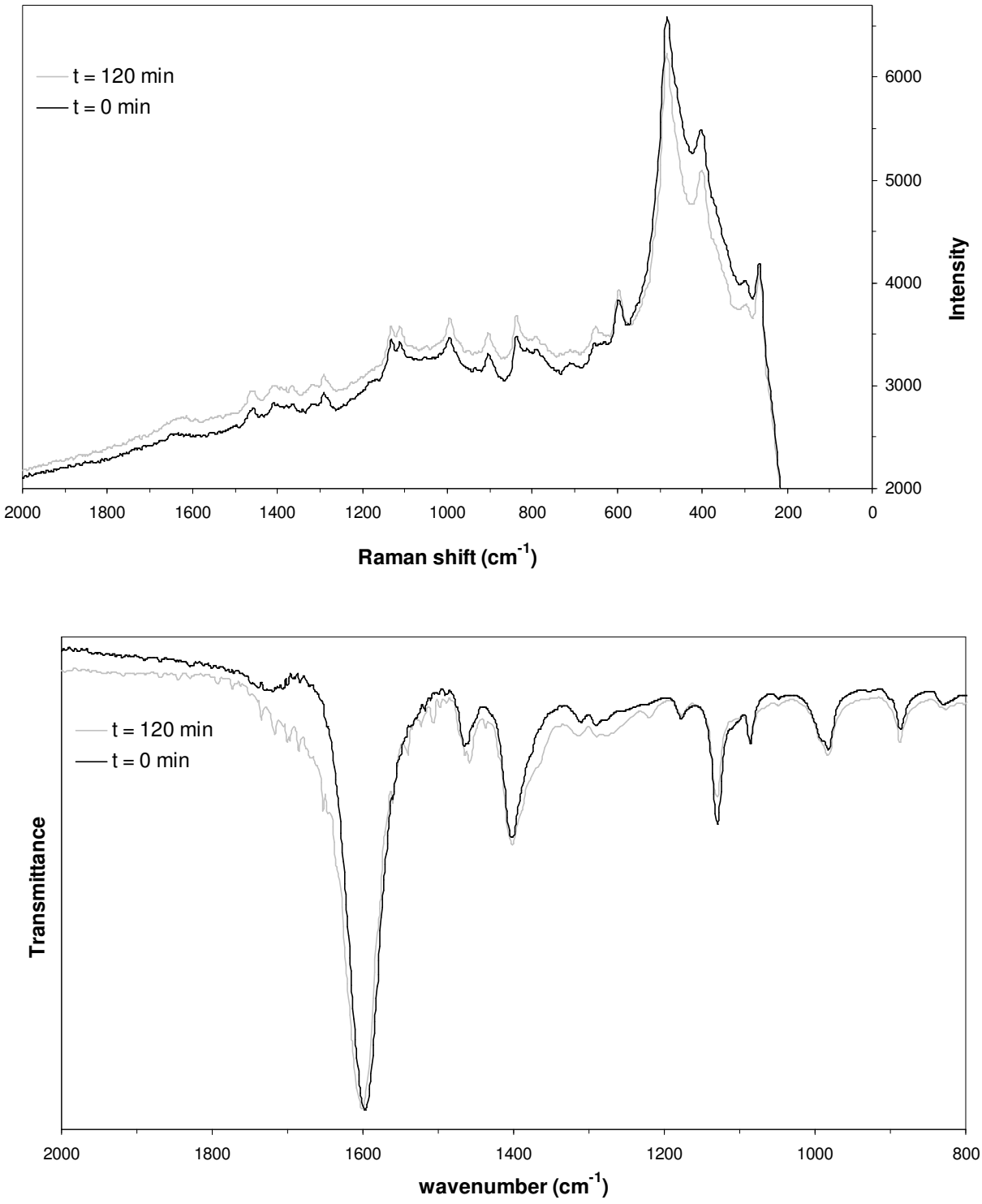
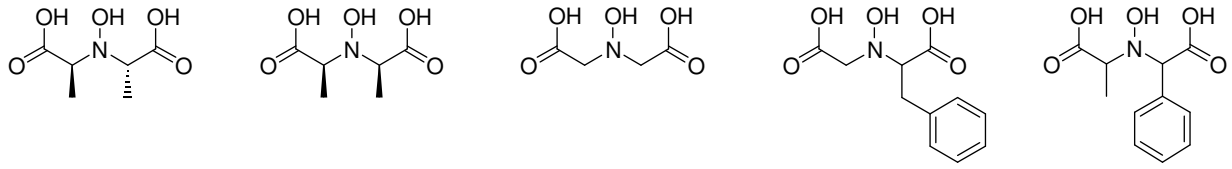


Figure 5.2. *Raman (top) and ATR-IR (bottom) spectra of dissolved dry amavadin after 0 and 120 minutes in water.*

The investigations of the natural amavadin (ligand **1**) were extended to other available amavadin analogues. The '*meso*-amavadin' (ligand **2**) displayed dynamic behaviour in water identical to the natural amavadin, while different behaviour was observed for analogues that have variations in their backbone substituents (ligands **3**–**5**).



1 2 3 4 5

Figure 5.3. *Ligand analogues used for investigations on the hydration of their vanadium complexes.*

The amavadin analogues with ligands **3** or **4** remained blue throughout drying. Their aqueous solutions were also blue and almost no changes in UV-VIS absorption were observed in time. The analogue prepared from ligand **5** was blue-purple after drying and it turned to blue during 45 minutes after dissolution in water (Figure 5.4).

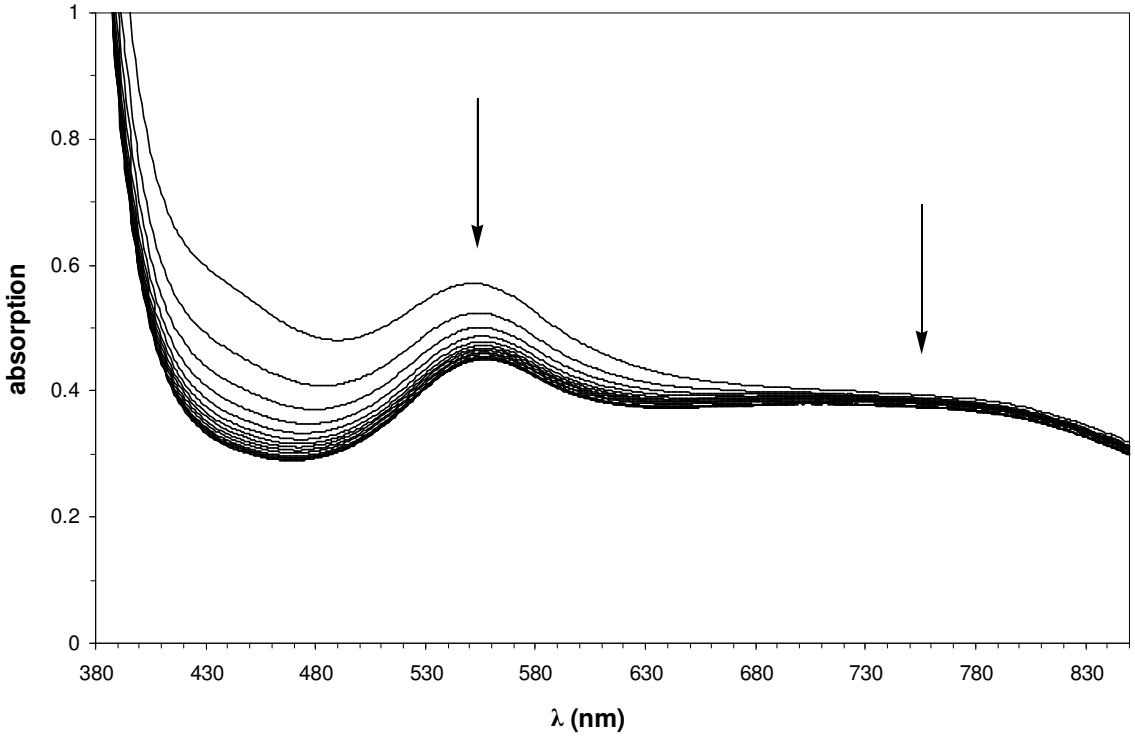


Figure 5.4. *Changes in UV-VIS during 45 minutes after dissolving the amavadin analogue of ligand* ***5*** *in water.*

5.2.3. Discussion and conclusions

Although it is known that amavadin is generally hydrated, no reports exist on amavadin that is completely void of water. We accomplished the dehydation of amavadin and confirmed the presence of amavadin·$0H_2O$ via a quantitative vanadium complexation by the ligand. Although the literature reports on amavadin show that amavadin can exist in colours that range from blue to purple, the fact of the different colours itself has never been mentioned. We are the first to

clearly distinguish between the blue and the purple forms of amavadin and we found a connection with the hydration of amavadin: hydrated amavadin is blue, dry amavadin is purple.

The nature of the observed colour changes in the complex is less trivial, but the various spectroscopic results shed some light on this. The absence of significant changes in the vibrational spectra (Raman, ATR-IR) demonstrated that the coordination bonds of the structure remain intact and that no other bonds are weakend or strengthened. This means that not only the octa-coordinated vanadium surrounding remained intact, but also that water molecules interact in a similar way with both complexes. The MS spectra confirmed that water does not react with amavadin, and neither are molecules formed that consist of more than one 'amavadin-unit'. The fact that changes in the molecule are only manifested in the UV-VIS domain indicates changes in the ligand field, i.e. changes in the electronic structure of the complex. We propose that the water serves as a sort of mortar in the structure and that removal of the water from the complex causes the ligand to reorganize its wrapping around the vanadium. This conformational change is then responsible for the observed changes in the ligand field. The presence of a high energy transition state to reach the other conformation would explain the slow reversion after contact with water.

5.3. Solubility of amavadin

The literature data on the solubility of amavadin mostly concern the hydrated amavadin. This compound is reported to be highly soluble in water, DMSO and DMF, while it is insoluble in most other solvents. From the point of application of amavadin as an homogeneous catalayst, solubility is an important factor. Therefore, we investigated if the differences between dry amavadin and hydrated amavadin are reflected in their solubilities.

5.3.1. Solubilities of hydrated and dry amavadin

In water, a distinction between hydrated and dry amavadin is not relevant. When other (dry) solvents were used, differences between the behaviour of wet and dry amavadin were observed (Table 5.1). Both the wet and the dry form are well soluble in DMSO and DMF and result in red solutions. The solubility of dry amavadin in methanol is also good, but decreases sharply for the higher alcohols. In most other solvents, both hydrated and dry amavadin appeared to be insoluble, including acetone, THF, acetonitrile and acetic acid. On the other hand, the solubilities in conjugated alcohols like benzyl alcohol, cinnamyl alcohol and 2-methyl-cinnamyl alcohol are comparable to those in methanol and ethanol. Although wet amavadin is moderately soluble in ethanol, heating of the mixture resulted in complete dissolution. This occurred under the formation of a red solution that has the same UV-VIS characteristics as the

red solution of dry amavadin in ethanol. Thus, amavadin converts to the red form upon displacement of water from the complex in solution as well as in the solid state.

Table 5.1. *Solubilities of wet and dry amavadin in a range of solvents.*[1,2)]

Solvent	Solubility of amavadin	
	hydrated amavadin	**dry amavadin**
water	+++	+++
DMSO	++	++
DMF	++	++
methanol	+	++
ethanol	0	+
1-propanol	--	0
2-propanol	--	0
1-butanol	--	-
benzyl alcohol	+	++
cinnamyl alcohol	--	+
2-methyl cinnamyl alcohol	--	+
benzaldehyde	- / 0	+
cinnamyl aldehyde	--	--
diethyl ether	--	--
ethyl acetate	--	--
dichloromethane	--	--
acetone	--	--
nitrobenzene	--	--
THF	--	--
acetic acid	--	--
acetonitrile	--	--
toluene	--	--

1) Electrospray-MS of all solutions showed the molecular mass of amavadin as the main signal.
2) If soluble, both forms result in red-purple solutions with the same UV-VIS characteristics

The above observations can be summarized as follows:

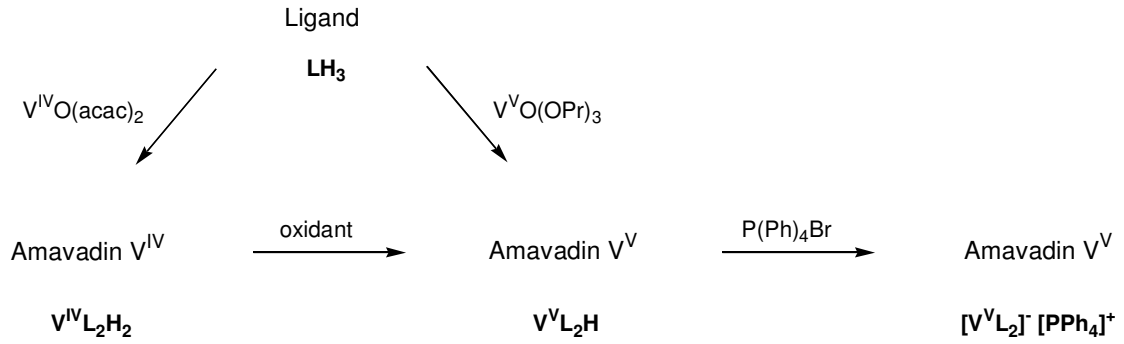
- In all solvents, dry amavadin is equally or more soluble than wet amavadin.
- When dry amavadin is soluble in a solvent other than water, a red or purple solution is formed.
- When wet amavadin is soluble in a solvent other than water, a blue solution is formed initially, which then turns red or purple.
- Amavadin is generally not soluble in solvents that are apolar *and* aprotic.

It was shown in section 5.2 that dry amavadin initially gives a red solution in water, that slowly turns blue. Solutions of dry amavadin in non-aqueous solvents remained red, which confirms the earlier proposition that water is involved in the observed colour changes in aqueous solution. It was furthermore shown that evaporation of a methanolic solution under vacuum gave the dry amavadin again, whereas slow evaporation in air yielded the hydrated amavadin as a blue solid.

The fact that alcohols are much more effective in solubilizing amavadin than for example acetone, acetonitrile and acetic acid points to a specific affinity of amavadin for alcohols (the affinity of vanadium for alcohols is generally known[14]). Apparently, amavadin's preference for water molecules is extended to alcohols, which implies similar roles of the hydroxy groups in both compounds. The solubility of dry amavadin in relatively apolar alcohols such as benzyl alcohol, cinnamyl alcohol and 2-methyl-cinnamyl alcohol can be the result of the conjugation in these molecules; the hydroxy group is more nucleophilic and can therefore provide more electron density to the proton(s) of amavadin.

5.4. Properties of oxidized amavadin

Because of the catalytic properties of V(V) as a Lewis acid, we separately studied the dynamic behaviour of oxidized amavadin. Scheme 5.1 shows how we obtained oxidized amavadin either as $V^{V}L_2H$ or $[V^{V}L_2][PPh_4]$. The de- and rehydration process and the reversible change in structure as found for amavadin were not observed for either one of these two complexes. However, we observed an irreversible reaction of $V^{V}L_2H$ in water (section 5.4.1). Investigations towards the solubility of both V(V) complexes revealed behaviour that was similar to that found for the V(IV) amavadin complex (section 5.4.2).

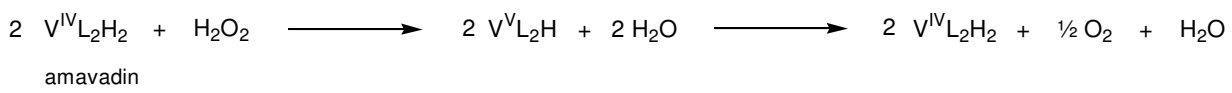


Scheme 5.1. *Applied routes to obtain the two forms of oxidized amavadin.*

5.4.1. Autoreduction of $V^{V}L_2H$ in water

Whereas the vanadium(IV) compound amavadin is very stable under atmospheric conditions, the oxidized species $V^{V}L_2H$ is only moderately stable: it appeared to revert back to the vanadium(IV) compound. This phenomenon was observed when amavadin was oxidized in water with $(NH_4)_2Ce(NO_3)_6$. After extracting the resulting $V^{V}L_2H$ species into dichloromethane as $[V^{V}L_2][PPh_4]$, a blue colour remained in the water layer, for which the $V^{IV}L_2H_2$ compound proved to be responsible. The reduction to V(IV) could not be observed once the V(V) is complexed by PPh_4Br, from which we concluded that the $[V^{V}L_2]$ anion is stabilized by the PPh_4-cation.

Autoreduction of oxidized amavadin was noticed earlier by Matoso *et al.*,[15] although these researchers used different experimental conditions. They performed the oxidation of amavadin with half an equivalent of H_2O_2, after which they observed that the solution reverted to the blue V(IV) amavadin under the formation of oxygen. The authors concluded that this is the result of the oxidization of water, and they considered this oxidation reaction as being part of a vanadium-catalyzed H_2O_2 decomposition reaction (catalase reaction, Scheme 5.2). However, no direct evidence for the oxidation of water was provided.[16]



Scheme 5.2. *Catalytic oxidation of water by amavadin as proposed by Matoso et al.*

The authors did not mention the possibility that the oxygen can be formed directly from the H_2O_2 as in most catalase reactions, which poses the question whether oxygen is also formed in the absence of H_2O_2. We could not observe any gas formation during the autoreduction after oxidation with $(NH_4)_2Ce(NO_3)_6$. Further investigations were therefore desired, but control over the $V^{V}L_2H$ species was difficult due to the *in situ* preparations with either $(NH_4)_2Ce(NO_3)_6$ or H_2O_2. Instead, solid $V^{V}L_2H$ was isolated after reaction of the ligand with $VO(OPr)_3$ in methanol (Scheme 5.1). Figure 5.5 shows the changes in UV-VIS absorption at time intervals of 2 minutes after dissolving the isolated $V^{V}L_2H$ in water. After twenty minutes, the solution had become blue and did not show evidence of gas formation. There was an isosbestic point at 661 nm; only the initial spectrum crossed slightly beyond this point. The absorption minimum moved from 458 to 476 nm and the absorption maximum from 521 to 560 nm. The final spectrum could be overlaid with that of amavadin (Figure 5.1), although a small discrepancy between the absorption minima remained (476 vs 452 nm). MS and NMR measurements showed that the product that is formed is indeed the V(IV) amavadin species. We also observed

that when the V^VL_2H powder was exposed to the air, the colour changed from red to blue within hours. These observations prove that reduction of V(V) to V(IV) takes place, but do not give clarity about the reducing agent. It is unlikely that water fulfills this role, considering the high redox potential of water and the absence of gas formation. Secondly, oxidation of the ligand is also unlikely, because several earlier CV-studies have shown that amavadin possesses a completely reversible one-electron redox couple between the V(IV) and V(V) oxidation states.[2,17] Moreover, the UV-VIS spectra can not account for the degradation of half of the amavadin species to fuel the oxidation of the other half (not even in the case of one oxidation per ligand, which results in a quarter of the amavadin being consumed). It seems, however, that the reduction of V(V) is water-assisted because the V^VL_2H species is stable in methanol. The observation that the methanol is not oxidized by the complex makes the oxidation of water even less probable. In conclusion, the observed autoreduction remains an elusive process for which we could not find an explanation.

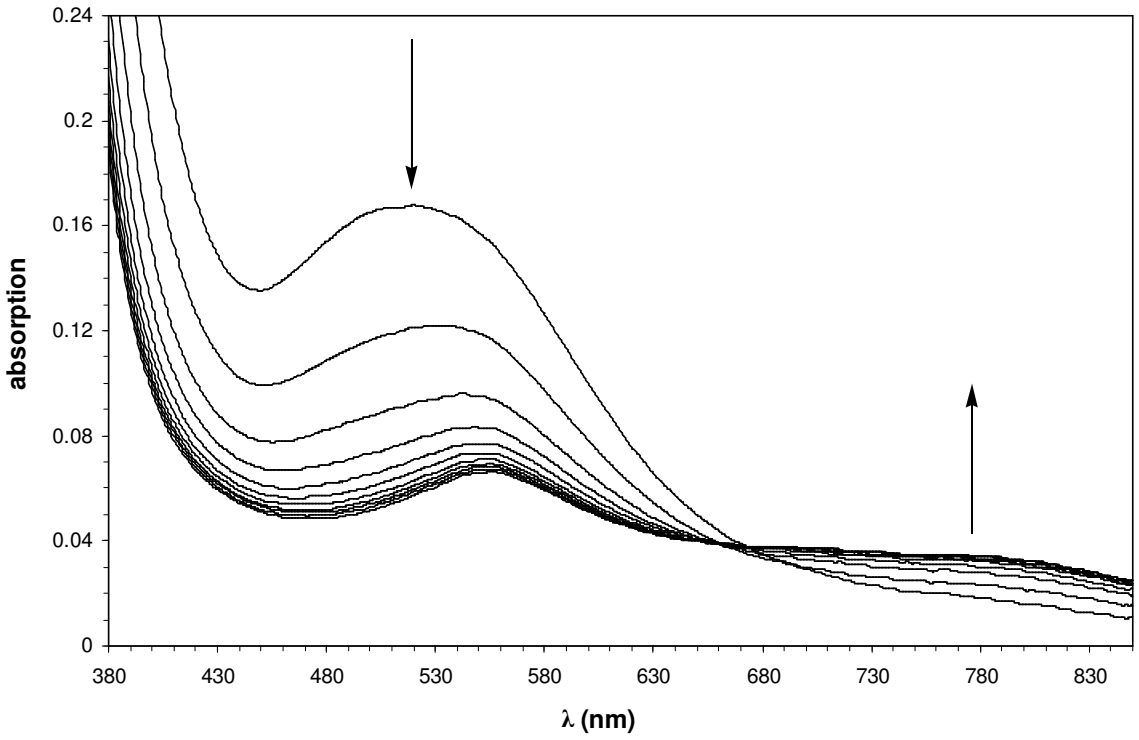


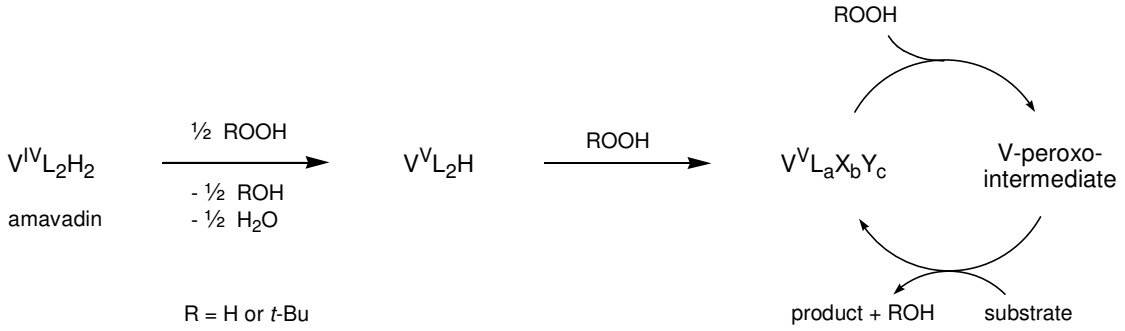
Figure 5.5. *Changes in UV-VIS during 20 minutes after dissolving V^VL_2H in water.*

5.4.2. Solubility of oxidized amavadin

The oxidized amavadin species V^VL_2H and $[V^VL_2][PPh_4]$ have largely the same solubility properties as those of the dry V(IV) amavadin. The only notable differences are found for $[V^VL_2][PPh_4]$ as it is also well-soluble in dichloromethane and acetonitrile.

5.5. Reaction of amavadin with H_2O_2 and TBHP

Chapter 6 describes the application of amavadin in oxidation catalysis using H_2O_2 or TBHP as the oxidant. To get insight into the nature of the vanadium species present in catalysis, we investigated the reaction of amavadin with H_2O_2 and TBHP. We propose that amavadin is first oxidized to V^VL_2H by half an equivalent of the peroxide (Scheme 5.3). Further reaction with the peroxide then leads to a species $V^VL_aX_bY_c$, that may be different from V^VL_2H; ligands X and Y can be oxo or peroxo ligands, respectively. The $V^VL_aX_bY_c$-species then enters the catalytic cycle of the oxidation, in which substrate oxidation can take place via a vanadium peroxo intermediate.



Scheme 5.3. *General scheme proposed for the application of amavadin in catalytic oxidations.*

Two types of conditions were applied for catalytic oxidations with amavadin: aqueous and anhydrous conditions. The behaviour of amavadin under the first is described in section 5.5.1, the behaviour under the latter in section 5.5.2.

5.5.1. Amavadin + H_2O_2/TBHP under aqueous conditions.

Amavadin was treated with aqueous solutions containing either 1, 3.5 or 100 equivalents of H_2O_2 or TBHP. The blue amavadin solutions immediately coloured red after the addition. Also, gas formation was observed, which confirmed the catalase activity of amavadin.

1 equivalent of H_2O_2 or TBHP. Both solutions coloured from the red V(V) to the blue V(IV) during 10 minutes, after which no peroxide could be detected. Thus, the catalase turnover frequencies of H_2O_2 or TBHP were approximately 0.1 min^{-1}. These processes produced UV-VIS spectra similar to those obtained when oxidized amavadin V^VL_2H was dissolved in water in the absence of H_2O_2 or TBHP (section 5.4.1).

3.5 equivalents of H_2O_2 or TBHP. Complete peroxide decomposition was only observed after 30 minutes in the TBHP solution and after 80 minutes in the H_2O_2 solution. Both solutions were purple at this stage, and did not change thereafter.

100 equivalents of H_2O_2 or TBHP. Both solutions remained red, and peroxide could still be detected after 48 hours.

Electrospray MS of all peroxide solutions showed the main presence of the $V^{V}L_2H$ species, while they showed no evidence for the existence of other species such as oxo- and oxoperoxovanadium compounds. Significant amounts of the $V^{IV}L_2H_2$ species were also observed in the case of 1 equivalent of H_2O_2 and in the case of 1 and 3.5 equivalents of TBHP. The observed reduction in these cases made it difficult to perform ^{51}V NMR measurements; the decreasing V(V) contents were confirmed by quantitative ^{51}V NMR measurements in time. Figure 5.6 shows the ^{51}V NMR spectra obtained after the addition of 1, 3.5 and 100 equivalents of H_2O_2, while Figure 5.7 shows the ^{51}V NMR spectrum after the addition of 100 equivalents of TBHP.

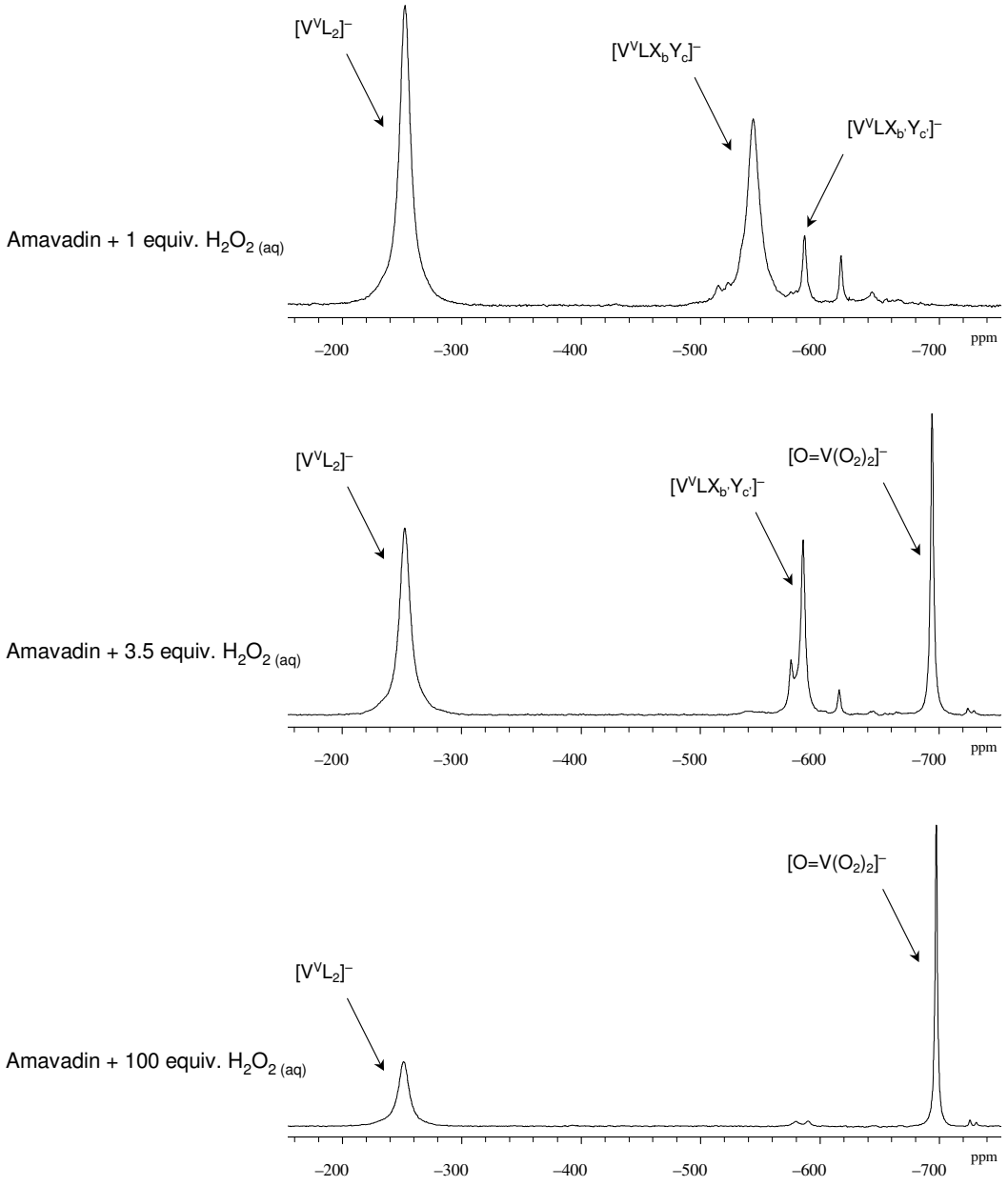
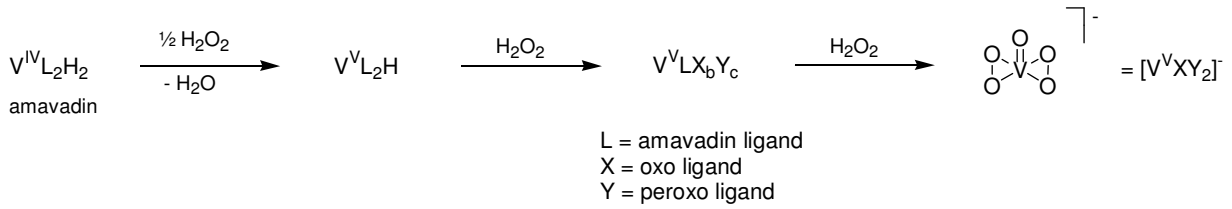


Figure 5.6. *^{51}V NMR spectra recorded directly after the addition of 1, 3.5 and 100 equivalents of H_2O_2 to amavadin.*

NMR after the addition of H_2O_2 (Figure 5.6). After the addition of 1 equivalent of H_2O_2, the ^{51}V NMR showed the presence of oxidized amavadin at –250 ppm and a second signal at –540 ppm (seemingly a composite signal). When 3.5 equivalents were added, the signal at –540 ppm disappeared while new signals were observed at –576, –586 and –696 ppm. When 100 equivalents were added, only the signal at –696 ppm remained. The latter represents the oxodiperoxovanadium(V) anion $[O=V(O_2)_2]^-$,[18,19] while the signals between –500 and –650 ppm presumably represent mono-ligand species $V^VLX_bY_c$. The molecular interpretation of the spectra in Figure 5.6 is given in Scheme 5.4. It picturizes the proposed progressive ligand dissociation upon H_2O_2 addition until the oxodiperoxovanadium(V) anion is formed. It can now be explained how an excess of H_2O_2 results in the observed decrease in catalase activity: the $[O=V(O_2)_2]^-$ anion is a weak base that requires protonation to be active as a catalase.[20] The significant H_2O_2 decomposition that was observed after the addition of 1 and 3.5 equivalents can be ascribed to the proposed mono-ligand species $V^VLX_bY_c$. These species are probably also formed as intermediates directly after the addition of 100 equivalents, where only an initial catalase activity is observed. This reasoning also explains the low yields in the catalytic oxidation reactions with this catalytic system (Chapter 6): the only species present are $[O=V(O_2)_2]^-$ and $[VL_2]^-$. The first is a poorly electrophilic anion and therefore a weak oxidizing agent, the latter is not a (per)oxo compound.



Scheme 5.4. *Proposed formation of vanadium species after the addition of H_2O_2 to amavadin.*

NMR after the addition of TBHP (Figure 5.7). In this case, amavadin was used that contains 80% of the (*S*,*S*)-ligand and 20% of the (*R*,*S*)-ligand. The solution with TBHP showed the presence of the diastereoisomers of oxidized amavadin between –220 and –270 ppm, while one other signal was present at –545 ppm. From the ^{51}V NMR it is not possible to conclude whether the ligand is still coordinated to vanadium. Presumably, ligand dissociation takes place, which can then give species $V^VL_aX_bY_c$ with a = 0 or 1 (L = amavadin ligand, X = oxo ligand and Y = OO*t*-Bu ligand). Apparently, none of the species in this mixture are active as a catalase. Furthermore, the low yields in the catalytic oxidation reactions with this catalytic system (Chapter 6) indicate that they are neither active as an oxidation catalyst.

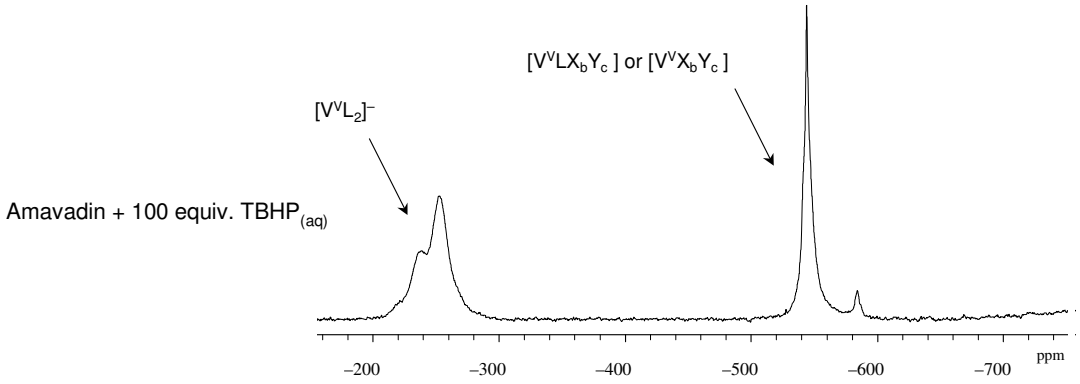


Figure 5.7. *^{51}V NMR spectrum recorded directly after the addition of 100 equivalents of TBHP to amavadin.*

5.5.2. Amavadin + H_2O_2/TBHP under anhydrous conditions.

The preparation of the active catalyst solution under anhydrous conditions involved the dissolution of water-free amavadin either in a 10% solution of H_2O_2 in ethyl acetate or in a 65% TBHP solution in heptane. For solubility reasons, the peroxides could only be added in an excess of >50 equivalents. Dilution of the resulting mixtures was generally not possible and the solutions therefore essentially contained the peroxide and the amavadin-catalyst; only a small amount of solvent remained (see Chapter 6). The rates of decomposition of H_2O_2 and TBHP under anhydrous conditions were much higher than in aqueous environment, but they also decreased after a few minutes. The water that was produced in the case of H_2O_2 formed a second layer and resulted in the mixture not being anhydrous anymore. Subsequently, the vanadium complex was extracted from the ethyl acetate into the new water layer, which resulted in a sticky heterogeneous mixture, that was not suitable for further analysis. The *tert*-butanol that was generated in the case of TBHP did not form a second layer and the mixture remained homogeneous. The ^{51}V NMR of the solution showed a broad signal between –200 to –300 ppm, while sharp signals appeared in the region of –520 to –620 ppm (Figure 5.1).

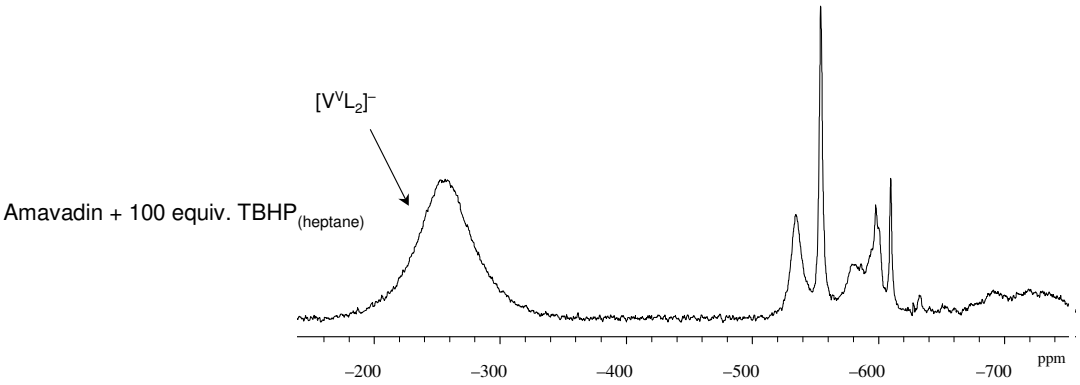


Figure 5.8. *^{51}V NMR of the solution of amavadin in a 65% TBHP solution in heptane.*

The broad signal most likely represents the oxidized amavadin, while the sharp signals are in the region where monoperoxo and monoperoxo-ligand species normally appear. Electrospray MS of the TBHP solution gave one signal, which corresponded to the $V^{V}L_2H$ species.

5.5.3. Conclusions and outlook

The results in this section show that the addition of a few equivalents of H_2O_2 to amavadin in water leads to the dissociation of one ligand, and that an excess H_2O_2 results in the loss of both ligands under the formation of the oxodiperoxovanadium(V) anion. This provides an explanation for the observed low catalase and oxidation activity of this mixture. In the case of 100 equivalents of TBHP in water, an unknown secondary vanadium species is formed. Analogous to the oxodiperoxovanadium(V) anion, this species also has a very low catalytic activity and is probably the result of irreversible ligand dissociation. The identity of the anhydrous mixtures of amavadin with H_2O_2 and TBHP remains largely uncertain.

Future studies in collaboration with the groups of L. Pettersson (University of Umeå, Sweden) and I. Banyai (University of Debrecen, Hungary), will be directed towards buffered aqueous solutions of vanadate, amavadin ligand and H_2O_2. Preliminary studies in both groups reveal that stable monoperoxovanadium mono-ligand species are formed after the addition of the amavadin ligand to a vanadate/H_2O_2 mixture near neutral pH. After our finding that amavadin itself is not stable in the presence of H_2O_2, the preliminary results of Pettersson and Banyai open new possibilities to obtain stable complexes of the amavadin ligand with vanadium in the presence of H_2O_2.

5.6. Experimental

The UV-measurements were performed at room temperature on a Cary-UV spectrophotometer. The Raman spectra were recorded using a Kaiser RXN dispersive system with a Peltier element-cooled Andor CCD camera for detection and a laser of 532 nm (60 mW). In these experiments, the measurements where done from the outside of the glass flask using a laser 10x objective. The resolution was about 2 cm^{-1} and 10 scans were accumulated with an exposure time of 10 s. The ATR-IR measurements were carried out at room temperature on a Perkin-Elmer 2000 Fourier transform spectrometer equipped with a DTGS detector. Spectra were recorded with a ATR accessory (MIRacle, Pike Technologies) with a ZnSe/Diamond crystal as the reflecting element. Spectral resolution was 4 cm^{-1} and 10 scans were accumulated for one spectrum. Mass spectroscopy was measured on a Micromass Quattro LC-MS spectrometer (Electron Spray Ionization). The C, H and N elemental analyses were performed at the Dr. Verwey Chemical Laboratory in Rotterdam and V elemental analyses were performed at the analytical department of the department of Biotechnology at the TU Delft using AAS. ^{51}V NMR spectra were recorded on a Varian VXR-400S (400 MHz) or a Varian Unity Inova 300 (300 MHz) instrument. Chemical shifts are expressed in parts per million (δ) relative

to the external reference $VOCl_3$. The preparation of the 10% solution of H_2O_2 in ethyl acetate and the 65% TBHP solution in heptane is described in Chapter 6.

5.7. Acknowledgements

The ATR and the Raman measurements were performed in the lab of Prof. B. Weckhuysen, University of Utrecht. We want to acknowledge Prof. Weckhuysen for giving us the possibility to perform the measurements, and we want to thank Fouad Soulimani and Kaisa Kervinen for their help during the measurements.

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AMAVADIN-CATALYZED (EP)OXIDATIONS

6

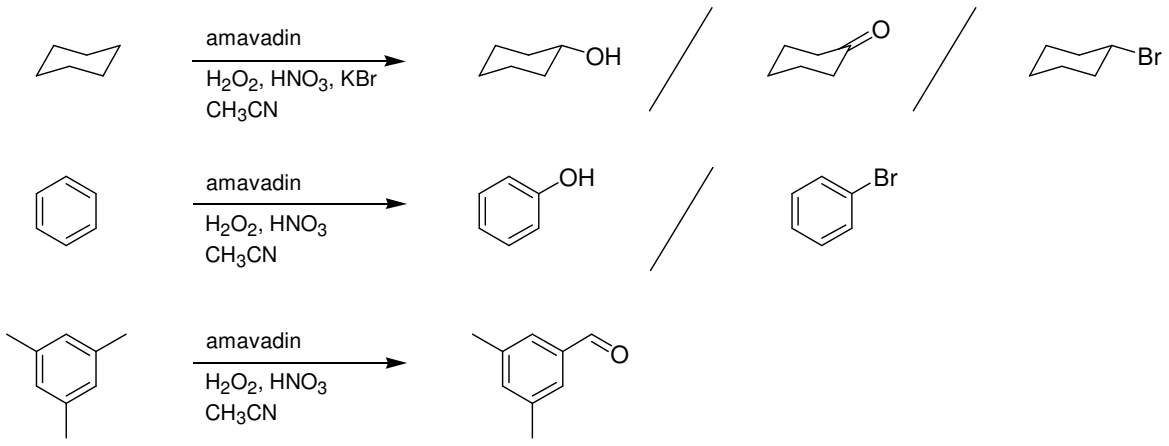
6.1. Introduction.

The application of vanadium in oxidation catalysis is a field of research that has received much attention in the last decades. A concise overview of homogeneous vanadium catalyzed oxidation reactions is given in Chapter 1, with a focus on the recent progress in asymmetric oxidations reactions. After the asymmetric synthesis of the amavadin ligand and the investigation of the properties of amavadin, curiosity has risen as to what extent amavadin can act as an oxidation catalyst. Moreover, amavadin bears two chiral ligands, which opens the possibility for application in asymmetric catalysis. In this respect, it is important to keep in mind that amavadin exists as an approximately equimolar mixture of Δ and Λ diastereoisomers,[1] even when it is prepared from an enantiopure ligand. There are numerous examples of non-linear effects in enantioselective catalysis and the possibility exists that such effects also appear in amavadin-catalyzed reactions. A positive effect would lead to a higher *ee* than the *ee* obtained with only one of the vanadium diastereomers ('asymmetric amplification'), and a negative effect would lead to an underestimation of the actual enantioselectivity of one of two vanadium diastereomers ('asymmetric depletion').[2,3]

A few applications of amavadin as an oxidation catalyst have been reported to date. These can be divided into three categories:

(1) Two-electron oxidations of saturated alkanes and aromatic compounds[4,5]

The applied catalyst in these cases is the Ca-salt of amavadin or its simpler acetate analogue (no methyl groups on the backbone). The catalytic system comprises the vanadium complex (0.2 mol%), hydrogen peroxide (H_2O_2 : V ratio ranges from 134–880) and nitric acid (HNO_3: V ratio ranges from 36–3610).

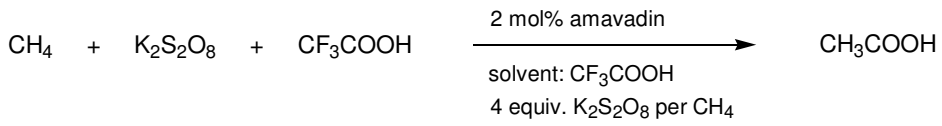


Scheme 6.1. *Amavadin-catalyzed two-electron oxidations of saturated alkanes and aromatic compounds.*

Using acetonitrile as a solvent, the halogenation (using KBr), hydroxylation and oxygenation of substrates such as cyclohexane, cyclooctane, benzene and mesitylene could be performed at room temperature in a reaction time of typically 6 hours. The yields were generally between 3 and 8% (Scheme 6.1). It was reported in the same papers that other vanadium compounds such as $VOSO_4$, V_2O_5 and $VO\{N(CH_2CH_2O)_3\}$ gave similar results. Rather than being some special property of amavadin, the observed catalytic activity is obviously due to the presence of vanadium in a form that can be generated from different catalyst precursors. These catalytic reactions likely proceed via a radical chain process involving hydroxyl radicals and can be characterized as Fenton chemistry.[6]

2) Conversion of methane into acetic acid[7,8]

Using the Ca-salt of amavadin or of its simpler acetate analogue, acetic acid was produced in yields of up to 29% from methane and the peroxodisulfate salt $K_2S_2O_8$ in a direct one-pot synthesis without the use of CO.[7] Typical conditions include conducting the reaction in CF_3COOH at 80 °C using 2 mol% of the vanadium catalyst and 4 equivalents of $K_2S_2O_8$ per CH_4 (Scheme 6.2). It was shown that methane is the carbon source of the methyl group of the acetic acid, while the carbon source of the carboxylic acid group is CF_3COOH. Mechanistic details are still unknown, although the report suggests that the carboxylation of methane involves the formation of the methyl radical and the methyl cation which would react with CF_3COOH.

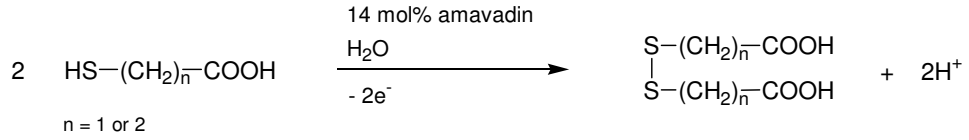


Scheme 6.2. *Amavadin-catalyzed conversion of methane into acetic acid.*

This catalytic system was further investigated by using (cyclo)alkanes such as cyclopentane, cyclohexane, pentane and hexane.[8] In all cases, mono-carboxylated products were formed. In case of the linear alkanes, regioisomers were formed, whose product distribution is indicative of a radical pathway. The authors claim that this new catalytic system has an advantage over the existing industrial processes of Monsanto and BP-Amoco, but they also state that a less expensive solvent, carboxylating agent and oxidant are still a requirement. After the first publication on this system, other scientists reported on a reaction where both carbons of acetic acid are derived directly from two methane molecules in a single reaction system, using a palladium catalyst in liquid sulfuric acid.[9]

3) Oxidation of thiols to the corresponding disulfides[10]

It was shown that in the presence of amavadin electrocatalytic oxidation of mercaptoacetic and mercaptopropionic acid to the corresponding disulfides takes place (Scheme 6.3). The reaction occurs through a mechanism involving Michaelis-Menten type kinetics, which suggests a possible biological role for amavadin. After preparative scale electrolysis in the presence of eight equivalents of the thiol, the disulfides were isolated in 85% yield. It appeared that the electrocatalytic effect was almost completely lost by replacement of the thiol group and/or the carboxylic group with other groups. The necessary presence of the carboxylic acid group two or three bonds away from the thiol group suggests a certain coordination of the substrate to the metal.



Scheme 6.3. *Electrocatalytic oxidation of mercaptoacetic and mercaptopropionic acid to the corresponding disulfides.*

The three reactions described above cover only part of the area of vanadium catalyzed oxidations. The aim was to further explore the properties of amavadin in homogeneous (asymmetric) catalysis, i.e. the oxidation of alcohols, the epoxidation of allylic alcohols and the oxidation of sulfides. The results of these investigations are described in this chapter. First, section 6.2 discusses the preparation of amavadin catalyst solutions, which appeared to suffer from limitations of the solubility of amavadin. Amavadin's properties in catalytic oxidations are discussed in sections 6.3 and 6.4.

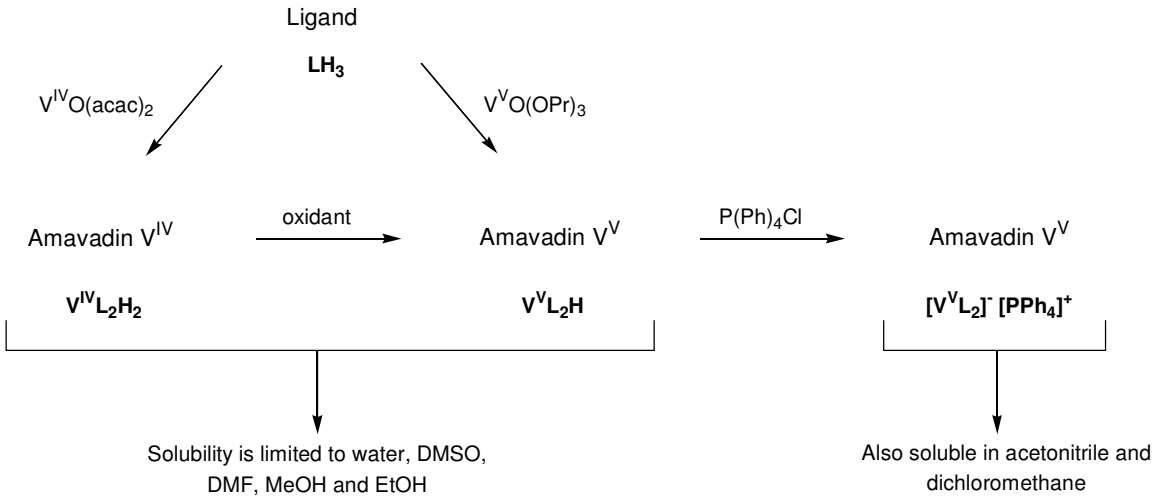
6.2. Catalyst preparation.

The investigation of amavadin catalyzed oxidations in a homogeneous solution required solubilization of amavadin. The preparation of amavadin catalyst solutions in either aqueous or anhydrous environment is described in this section.

6.2.1. Aqueous conditions

Amavadin is protic and polar, which results in a high solubility in water. This makes it easy to perform reactions using aqueous peroxide solutions. Moreover, when an aqueous-organic two-phase solvent system is prepared, the amavadin stays in the aqueous layer, while the substrate and/or the product is in the organic layer. This is an advantage when the product needs to be separated from the catalyst. Another consequence of amavadin's protic and polar properties, however, is that its solubility is limited to water, DMSO, DMF and the lower alcohols

methanol and ethanol (see also Chapter 5). The solubility remains the same when amavadin is oxidized to its +5 oxidation state. In the presence of the PPh_4^+ cation, the oxidized amavadin is soluble in dichloromethane and acetonitrile (this compound can be isolated as $[V^VL_2][PPh_4]$). The solubility of the free ligand is comparable to the solubility of amavadin, and an *in situ* preparation of amavadin from the ligand and a vanadium precursor is therefore also limited to water, DMSO, DMF, methanol and ethanol (Scheme 6.4).



Scheme 6.4. *Overview of the solubility behaviour of the different amavadin species.*

After the discovery of these solubility properties of amavadin, the investigation of amavadin catalyzed oxidations was started using aqueous solutions of hydrogen peroxide (H_2O_2) or *tert*-butyl hydroperoxide (TBHP). The use of the other possible solvents is either unattractive (DMSO, DMF) or is inappropriate because of interference with the reaction conditions (DMSO, methanol, ethanol).

6.2.2. Anhydrous conditions

When it is desired to carry out oxidations in the absence of water, it is common practice to use an organic peroxide that is dissolved in a (dry) organic solvent. For example, a 65% TBHP solution in heptane was easily obtained by extracting commercially available 70% aqueous TBHP into heptane, followed by drying on $MgSO_4$ (although the most common method to prepare such TBHP solutions involves Dean-Stark distillations,[11] but this is more hazardous). With a similar procedure, an anhydrous solution of 10% of H_2O_2 in ethyl acetate[12] was obtained, although the water that is inherently formed from H_2O_2 during the oxidation will lead to a non-anhydrous reaction mixture at a later stage. Despite the fact that none of the forms of amavadin as shown in Scheme 6.4 are soluble in heptane or ethyl acetate to any extent, we investigated the reaction of solid amavadin with a 65% TBHP solution in heptane or a 10% H_2O_2 solution in ethyl acetate. In this context of anhydrous solutions, it is important to note

that amavadin is generally prepared as its hydrate, but that water-free amavadin can be obtained after extensive drying (see Chapter 5). The hydrated amavadin (a blue solid) appeared to be insoluble in both peroxide solutions, but the water-free amavadin (a purple solid) gave red homogeneous mixtures in both cases after good stirring. In this way, solutions were obtained that essentially contain the oxidant (i.e. TBHP or H_2O_2) and the amavadin-catalyst; only a small amount of solvent remains. These catalyst solutions were diluted; DMSO, DMF and acetonitrile were successful solvents, and also the ionic liquids [tri(*n*-hexyl)tetradecylphosphonium][$N(SO_3CF_3)_2$], [Bmim][BF_4], [Bmim][$N(CN)_2$] and [3-Me-1-oct-pyridinium][BF_4] gave homogeneous solutions. When other solvents were used, a red precipitate was obtained. A drawback of this way of catalyst preparation is that an *in situ* complexation of vanadium to the ligand is not possible. This makes it impossible to prepare the active catalyst with different ligand to vanadium ratios, a variable which has proven to be important for the optimization of other catalytic systems, e.g. the vanadium catalyzed epoxidation of allylic alcohols.[13] Nevertheless, the different catalyst dilutions and the undiluted solution offered a good basis for investigations on the catalytic action of amavadin. In addition, the substoichiometric amount of heptane that is present in the reaction mixtures with TBHP can be used as an internal standard in GC-analysis.

6.3. Catalase activity of amavadin[14]

6.3.1. Aqueous conditions

When H_2O_2 is added to an aqueous solution of amavadin in the absence of other substrates, decomposition of H_2O_2 and TBHP takes place.[15] It has been shown in Chapter 5 that the initial turnover frequencies are approximately 0.1 min^{-1} and that the rate of decomposition slows down after a few turnovers; complete decomposition of 100 equivalents of both peroxides is not reached after 48 hours.

6.3.2. Anhydrous conditions

Under the anhydrous conditions with only a small amount of solvent, the decomposition of H_2O_2 and TBHP is much faster than in aqueous environment, which can be explained 1) by reasoning that water inhibits the catalyst and 2) by the fact that the solutions are very concentrated. After the preparation of the H_2O_2 solution in ethyl acetate, a high initial rate of H_2O_2-decomposition is observed and the rate slows down immediately. The water that is formed during this process separates from the ethyl acetate solution and absorbs its red colour. This reveals transfer of the amavadin from the ethyl acetate solution to the newly-formed water layer, which is in line with amavadin's preference for water as a solvent. When molecular sieves are added to scavenge the water in this system, the decomposition reaction proceeds

vigorously. The red mixture stays homogeneous, releasing much gas and heat and the decomposition of 200 equivalents of H_2O_2 is complete within one minute. These observations show that water strongly inhibits the catalase activity of amavadin. On the other hand, the mixture of TBHP in heptane also exhibits slower speed of TBHP-decomposition, which points to the inhibition by *tert*-butanol generated during the process. Decomposition of 100 equivalents of both peroxides is not completed after 48 hours.

6.4. Oxidation reactions catalyzed by amavadin.

6.4.1. Aqueous conditions

The oxidation of alcohols in (unbuffered) aqueous solutions was investigated using either H_2O_2 or TBHP in a bi-phasic system of water and the alcohol. The reaction was run for 24 hours at RT, using 10% molar excess of the oxidant and 2.5 mol% of amavadin. The substrates tested were benzyl alcohol, cinnamyl alcohol, 1-octanol and 2-octanol. Only in the case of the two conjugated alcohols a significant catalytic conversion to the corresponding aldehyde was observed, TBHP performing better than H_2O_2. Longer reaction times gave marginally increased yields (while peroxide was still proven to be present). The corresponding carboxylic acids could not be detected, neither could the epoxide of cinnamyl alcohol. The substrate 2-octanol did not show conversion at all. The values of the turnover numbers (TON) and yields of aldehyde or ketone are given in Table 6.1.

Table 6.1. *Amavadin-catalyzed oxidation of alcohols to their respective aldehydes/ketones, using H_2O_2 or TBHP as the oxidant*[1)].

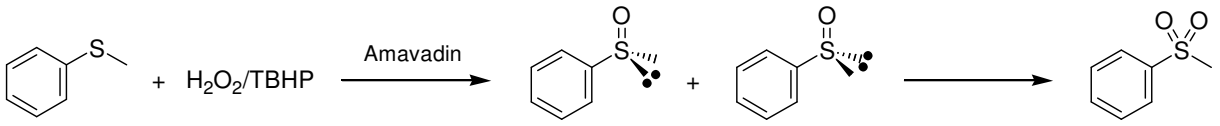
Entry	Substrate	TON[2)]		Yield[3)]	
		H_2O_2	TBHP	H_2O_2	TBHP
1	benzyl alcohol	2	8	5	20
2	cinnamyl alcohol	7	15	18	38
3	1-octanol	0	1	0	3
4	2-octanol	0	0	0	0

1) Conditions: bi-phasic system of water and alcohol, 2.5 mol% of amavadin, T = RT, reaction time = 24 h.
2) TON (turnover number) is defined as the number of moles of aldehyde or ketone that is formed per mole of catalyst.
3) Yield of aldehyde or ketone in %.

No improvement was observed when the reaction was carried out at a higher temperature (80 °C) or when a one-phase system was created by the addition of acetonitrile. The non-reactivity of aliphatic alcohols on the one hand and the oxidation of benzyl alcohol on the other hand is in accordance with the results of Reis et al.[4,5] They showed that the oxidation of alkanes stops at the alcohol stage, and that the oxidation of mesitylene leads to 3,5-dimethylbenzaldehyde via

the intermediate alcohol (and also in general, the C-H bond of the carbinol in aliphatic alcohols is stronger than that in benzylic alcohols[16]).

The oxidation of thioanisole was also carried out under one-phase or bi-phasic conditions, using either amavadin or its oxidized equivalent $[V^{V}L_2][PPh_4]$ (Scheme 6.5).



Scheme 6.5. *Amavadin-catalyzed oxidation of thioanisole.*

Catalytic turnover was only observed in the one-phase acetonitrile/water system and only by applying amavadin in the +4 oxidation state. When 1.0 equivalent of H_2O_2 or TBHP was used (added as one portion prior to the reaction), full conversion of the thioanisole was reached within 8 hours, yielding the sulfoxide and the sulfone in a ratio of 10:1. The use of 4 equivalents of H_2O_2 or TBHP yielded an approximately equimolar mixture of the sulfoxide and the sulfone after 48 hours (Table 6.2). In none of the reactions could stereoselectivity or kinetic resolution be observed.

Table 6.2. *Results on the oxidation of thioanisole[1] using amavadin in the +4 oxidation state ($V^{IV}L_2H_2$).*

conditions	equiv. TBHP or H_2O_2	conversion	% sulfoxide	% sulfone
2-phase (H_2O/dichloromethane)	1	0	-	-
	4	0	-	-
1-phase (H_2O/acetonitrile)	1	100	90	10
	4	100	50	50

1) Conditions: 2.5 mol% of amavadin, T = RT, reaction time = 8 h.

The results obtained with H_2O_2 can partly be explained on the basis of the results reported in Chapter 5. It was shown with ^{51}V NMR that reaction of amavadin with an excess of H_2O_2 yields the oxodiperoxovanadium(V) species as the main species, which is known to be a relatively weak oxidizing agent.[17] Moreover, being a strong acid, it exists as a poorly electrophilic anion in aqueous solution. This explains the formation of significant amounts of sulfone, because oxodiperoxovanadium(V) species can also accept the electrophilic sulfoxide as a substrate.

Although the results on the alcohol oxidation and the sulfoxidation prove that amavadin is active as an oxidation catalyst, the results in terms of activity and enantioselectivity are disappointing. After the observation that the catalase activity of amavadin increases under anhydrous conditions, higher activity of amavadin in oxidations was expected under these conditions. Therefore, further investigations were directed towards the anhydrous system.

6.4.2. Anhydrous conditions

The oxidation of benzyl alcohol, cinnamyl alcohol and 1-heptanol with H_2O_2 or TBHP was performed under the anhydrous conditions with only a small amount of solvent. The effect of diluting this mixture with ionic liquids (IL) was also investigated. The results of the oxidations are shown in Table 6.3.

Table 6.3. *Results of the amavadin-catalyzed oxidation of benzyl alcohol, cinnamyl alcohol and 1-heptanol with H_2O_2 or TBHP under anhydrous conditions.*

entry	solvent	T (°C)	% amavadin	equiv. oxidant	reaction time (h)	conversion	yield	yield
	oxidation of benzyl alcohol					*alcohol*	*aldehyde*	*acid*
1	no solvent	40	1.5	2.5 H_2O_2	4 18	17 17	8 8	5 5
2	$[PR_4][Tf_2N]$ [1]	40	1.5	2.5 H_2O_2	5 20	8 9	8 9	2 2
3	other IL [2]	40	1.5	2.5 H_2O_2	4 18	<6 <8	<5 <6	0 <1
4	no solvent	40	1.5	2.5 TBHP	4 18	97 97	2 3	89 93
5	$[PR_4][Tf_2N]$ [1]	40	1.5	2.5 TBHP	5 20	62 87	27 13	36 73
6	other IL [2]	40	1.5	2.5 TBHP	4 18	<3 <9	<2 <8	0 0
	epoxidation of cinnamyl alcohol					*alcohol*	*epoxide*	*aldehyde*
7	no solvent [3]	40	1.5	1.2 H_2O_2	2 8	42 42	3 3	9 9
8	no solvent [3]	40	1.5	1.2 TBHP	2 8	96 96	90 86	5 5
	oxidation of 1-heptanol					*alcohol*	*aldehyde*	*acid*
9	no solvent or IL [2]	80	2.0	2.5 H_2O_2	5 20	0 0	0 0	0 0
10	no solvent or IL [2]	80	2.0	2.5 TBHP	5 20	0 <2	0 0	0 0

1) $[PR_4][Tf_2N]$ is [tri(*n*-hexyl)tetradecylphosphonium][$N(SO_3CF_3)_2$].
2) The other ionic liquids (IL) tested were [Bmim][BF_4], [Bmim][$N(CN)_2$] and [3-Me-1-oct-pyridinium][BF_4].
3) The use of ionic liquids led to hetereogeneous mixtures from which no acceptable mass balances could be obtained.

Oxidations in ionic liquids[18]

In general, the best results are obtained when the undiluted catalyst mixture is used. Only in the case of benzyl alcohol oxidation did dilutions with ionic liquids give accurate and reproducible results. In the epoxidation reactions, the ionic liquids seem incompatible with the reaction conditions as the reaction suffered from the formation of precipitates leading to mass balances of <60%. In none of the attempted oxidations of 1-heptanol could oxidation products be observed and the application of ionic liquids did not make a difference in this.

Use of H_2O_2 *vs* TBHP

The most striking difference between the use of H_2O_2 and TBHP are the constantly low conversions in the reactions with H_2O_2. When the conversions in these reactions are followed in time, it is seen that the catalyst becomes inactive already after conversions of less than 5%. For better understanding, the relative rates of product formation in the oxidation reactions using H_2O_2 in aqueous and non-aqeous media are summarized in Table 6.4.

Table 6.4. *Relative rates of product formation in the oxidation reactions using H_2O_2 and substrate (S) in aqeous and non-aqeous media, eventually in the presence of molecular sieves (MS).*

Solvent	Equation of the amavadin-catalyzed reaction		Relative rates of product formation
Water	$H_2O_2 + S$	$\rightarrow$ ½O_2 + H_2O	Very slow
		$\rightarrow$ SO + H_2O	Very slow
Ethyl acetate	$H_2O_2 + S$	$\rightarrow$ ½O_2 + H_2O	Initially fast, then decreasing
		$\rightarrow$ SO + H_2O	Initially fast, then decreasing
	$H_2O_2 + S + MS$	$\rightarrow$ ½O_2 + H_2O	very fast, vigorous
		$\rightarrow$ SO + H_2O	no product formation

The oxidation and the decomposition reactions are very slow in water and fast in ethyl acetate. In the latter, however, the reaction slows down because of the water that is inevitably formed during the oxidation. Attempts to scavenge the water with molecular sieves after the addition of substrate led to a very fast decomposition of H_2O_2 while no substrate oxidation took place. Oxidations with TBHP have the advantage that the reaction conditions can be maintained anhydrous, and it was seen that the reaction rates with TBHP remained high at conversions exceeding 50%. However, it must be kept in mind that the *tert*-butanol that is formed from TBHP is also capable of inhibiting oxidation reactions.[19]

Oxidation of benzyl alcohol

In the oxidation of benzyl alcohol, benzaldehyde was further oxidized to benzoic acid. Diluting the catalyst with the different ionic liquids led to a decrease in reaction rate. Only dilution with [tri(*n*-hexyl)hexyltetradecylphosphonium][$N(SO_3CF_3)_2$] gave good conversion, while dilution with more hydrophilic ionic liquids led to conversions below 10%. This can be explained by the fact that the hydrophobic [tri(*n*-hexyl)hexyltetradecylphosphonium][$N(SO_3CF_3)_2$] is the only ionic liquid with a non-coordinating anion and therefore does not block electrophilic species that are catalytically active.

Epoxidation of cinnamyl alcohol

The amavadin-catalyzed epoxidation of cinnamyl alcohol is fast and efficient, which makes it the most successful of the investigated amavadin catalyzed oxidations. Unfortunately, no enantioselectivity was observed in these epoxidations. When the temperature of the reaction was decreased to −10 °C, no differences in terms of (enantio)selectivity were seen in (chiral) HPLC or GC. To put these results into a context, the catalytic activity of two other V(IV) compounds was tested for comparison: $VO(acac)_2$ and $VO(OAc)_2$. These compounds were subjected to the same process of catalyst preparation and then applied in the epoxidation under the same conditions as amavadin. $VO(acac)_2$ is soluble in various organic solvents, and its solubility is generally no issue in catalyst preparation. $VO(OAc)_2$, on the other hand, is a very fine powder (dust hazard[20]!) that is not soluble in water or any other solvent, except concentrated acid. It appeared that both $VO(acac)_2$ and $VO(OAc)_2$ easily dissolved in the 65% TBHP-solution in heptane under the formation of a red homogeneous mixture. The catalytic epoxidation of cinnamyl alcohol using equal loadings of the three vanadium catalysts was then followed in time at 25 °C (Figure 6.1).

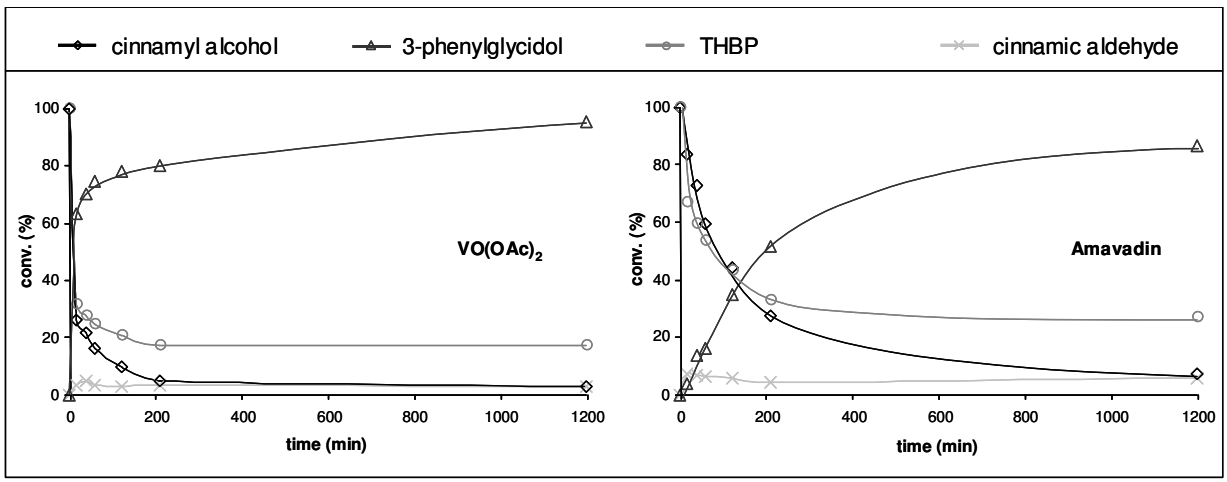
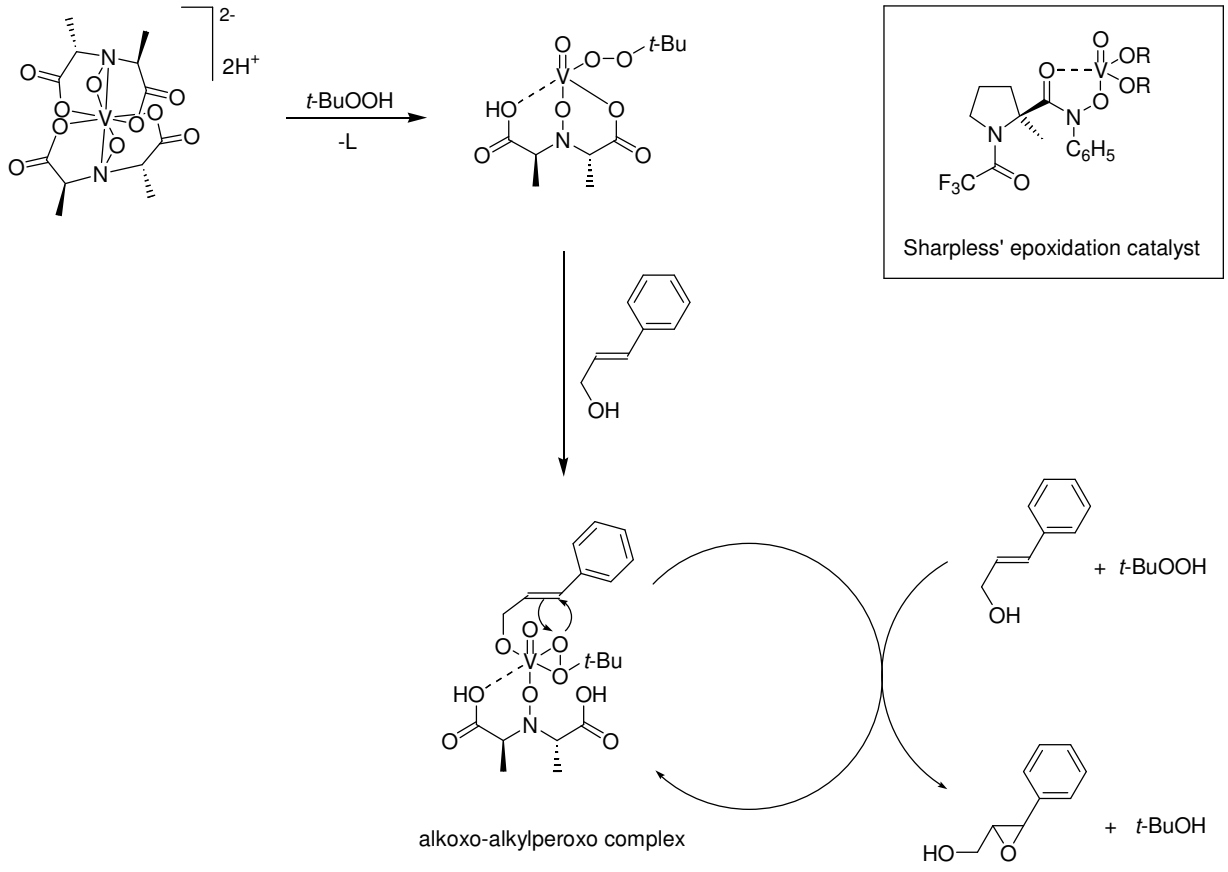


Figure 6.1. *Catalytic epoxidation of cinnamyl alcohol vs. time for two vanadium catalysts.*

The profiles of the reactions catalyzed by $VO(acac)_2$ and $VO(OAc)_2$ appeared to be almost identical, therefore Figure 6.1 shows only the results obtained with $VO(OAc)_2$ and with amavadin. The graphs show that after 20 hours of reaction, 98% epoxide yield is obtained with $VO(OAc)_2$ and 88% epoxide yield with amavadin. The reaction with $VO(OAc)_2$ shows a high and steady initial speed, that slows down abruptly after a certain time. This is due to inhibition by *tert*-butanol[19] but this effect is less pronounced in the amavadin-catalyzed reaction. When the reactions are carried out in the presence of extra *tert*-butanol,[21] a decrease in the reaction rate is observed for all three catalysts.

The observation that the reaction is significantly slower when amavadin is used rather than $VO(acac)_2$ or $VO(OAc)_2$ is an indication that the active catalysts are of different composition. It is known that the acetylacetonate (=acac) and the acetate ligands dissociate[22] from vanadium after reaction with TBHP in anhydrous and non-polar solvents, and that the actual oxidant in allylic epoxidations is an alkoxo-alkylperoxovanadium complex.[23]



Scheme 6.6. *Proposed generation of an active catalyst from amavadin and the proposed catalytic cycle.*

The amavadin ligand coordinates much stronger to the vanadium than the acac and the acetate ligands, and it is therefore likely that the ligand of amavadin still has a significant interaction

with the metal during the epoxidation. The different reaction profiles in Figure 6.1 reflect this difference in ligand affinity. It is, however, difficult to clarify the nature of the active species that is generated from amavadin. The catalytic cycle of vanadium-catalyzed epoxidation reactions usually includes vanadyl species. In the case of the triprotic amavadin ligand, this would require one ligand to be released from the metal, while the initial V-nonoxo compound becomes a vanadyl species. One possibility of generating such species in a catalytic cycle is shown in Scheme 6.6. The *N*-O ligand is bound to the vanadium in a fashion that bears resemblance with the chiral hydroxamate complex that is described by Sharpless and coworkers[24,25] for vanadium catalyzed asymmetric epoxidations.

Anhydrous epoxidation: molecular sieves

Besides inhibition by *tert*-butanol, inhibition can also be due to diols that are formed by incidental (catalytic) opening of the epoxide due to the presence of water. In addition, it is known from Sharpless' epoxidation procedure that water can interact both reversibly and irreversibly with the catalyst, and that this can be prevented by the addition of molecular sieves of the A-type.[26] Therefore, instead of carrying out the reaction under normal atmosphere in a closed vessel, the catalytic epoxidation reaction with amavadin was carried out under strictly anhydrous conditions, i.e. under nitrogen and in the presence of molecular sieves. Comparison of both reactions revealed no significant differences in the yields and the conversion profiles in time.

Catalyst loading

When the catalyst loading in the epoxidation reaction at 40 °C was 0.05 instead of 1.5 mol% of amavadin, the maximum epoxide yield was also approaching 90%. The reaction time in this case was 40 hours instead of 2 hours.

Application of oxidized amavadin in the presence of PPh_4^+

Scheme 6.4 shows that oxidized amavadin is moderately soluble in dichloromethane and acetonitrile when it has a PPh_4^+ counterion. It was investigated if anhydrous oxidations can be performed with the $[VL_2][PPh_4]$ complex, using the 65% TBHP solution in heptane or the 10% H_2O_2 solution in ethyl acetate. Unfortunately, no conversions of the substrates of Table 6.3 were measured when this catalyst was used.

Recycling of the catalyst

An advantage of amavadin as a catalyst over simple vanadium salts could be its stability, and we made attempts to recycle the catalyst in the oxidation and epoxidation reactions. Precipitation of the catalyst with solvent or ionic liquid allowed the separation of the product,

but it was not possible to obtain an active and homogeneous catalyst solution for the next run. Moreover, water was not applicable during the product extraction from the ionic liquids, because of the necessity to maintain the catalyst in an anhydrous environment.

Application of titanium-substituted amavadin

The only metal analogue of amavadin that was synthesized in this project is the titanium-substituted amavadin (Chapter 4). Encouraged by the good results obtained with amavadin in the catalytic epoxidation and bearing in mind the known activity of titanium complexes in catalytic asymmetric epoxidation (see Chapter 1), it was attempted to perform catalytic oxidations with the titanium-substituted amavadin. Like amavadin, this compound is also insoluble in organic media. Therefore, it was attempted to dissolve the complex in the 65% TBHP solution in heptane analogously to the procedure that was followed for amavadin. Unfortunately, the complex did not dissolve at all, nor was the resulting mixture active in the oxidation reactions. Also under aqueous conditions, no catalytic activity was observed. This is in fact in accordance with the observation made by Garner[27] that the titanium-substituted amavadin is redox inactive. It also supports the idea that the oxidation to the +5 oxidation state in the case of amavadin prior to the reaction is of primary importance.

6.4.3. Conclusions

The high solubility of amavadin in water and the low solubility in many other solvents results in a small window of application in oxidation catalysis. Oxidations in water occur only for a limited number of alcohols and in low yields. The amavadin-catalyzed decomposition of H_2O_2 and TBHP is strongly inhibited by water, which can be a result of amavadin's hydrophilic nature and its preference to retain water molecules in its structure.[28] Oxidation of thioanisole yields the sulfoxide and significant amounts of the sulfone. None of the oxidations in water occurred stereoselectively. The measured catalytic activities of amavadin in aqueous solution are similar to those obtained for other vanadium complexes[4,5] that proceed via radicals (Fenton chemistry).

Homogeneous oxidations in non-aqueous and aprotic environment require thouroughly dried amavadin and are essentially limited to systems that contain almost no solvent. Dilution of the reaction with different kinds of solvents, including some ionic liquids, do not lead to better performance. Best results are obtained using TBHP as the oxidant. Epoxidation of cinnamyl alcohol occurs in high conversion and high yield. Despite the proposed interaction of vanadium with the chiral ligand, the reaction is not stereoselective. Oxidation of benzyl alcohol leads almost exclusively to benzoic acid, and amavadin is not active in the catalytic oxidation of

aliphatic alcohols. The use of H_2O_2 seems to be incompatible with amavadin: the oxidations are very slow in the presence of water, while scavenging the water favours the H_2O_2 decomposition to such an extent that substrate oxidation does not take place anymore. The use of $[VL_2][PPh_4]$ does not lead to an active catalyst, also not in the reactions where the application of amavadin in its +4 oxidation state does show activity.

6.5. Experimental

Typical procedure for the amavadin catalyzed oxidations in aqueous environment. A 5 mM stock solution of amavadin in water was prepared. To a 20 mL glass tube with screw cap and septum, containing 2 mL of the stock solution (0.01 mmol of amavadin), was added the aqueous peroxide solution. The substrate (0.4 mmol) was added to the resulting red solution, after which the mixture was strirred at RT for the desired period of time. After extraction of the organic phase with 10 mL of diethyl ether and the addition of 0.20 mmol of undecane (internal standard), the mixture was quantitatively analyzed by GC.

Typical procedure for the amavadin catalyzed oxidations in anhydrous environment. To a 10 mL glass tube with screw cap containing 3 mg (7.5 μmol) of the dried amavadin was added 250 mg of a 65 m% TBHP/heptane solution (1.8 mmol of TBHP). This mixture was stirred until the amavadin was completely dissolved as a deep red solution. In cases where a solvent/ionic liquid/additive was used, it was added at this stage, i.e. directly after the catalyst preparation. Subsequently, 84 mg (0.75 mmol) of ISTD (chlorobenzene) and 1.5 mmol of the substrate were added. This mixture was stirred and the work-up of samples for GC was performed over a plug of silica in a Pasteur pipette with diethyl ether as an eluent. The catalyst and the ionic liquid (if used) resided on the silica.

Extraction of aqueous TBHP into heptane: A 250 mL solution of 70 m% TBHP in water is saturated with NaCl and then extracted into 90 mL of heptane in an extraction funnel. After drying over $MgSO_4$, the TBHP solution in heptane is ready for use.

Extraction of aqueous H_2O_2 into ethyl acetate: A 120 mL solution of 35 m% H_2O_2 in water is saturated with NaCl and then extracted into 50 mL of ethyl acetate in an extraction funnel. After drying over $NaSO_4$, the H_2O_2 solution in heptane is ready for use.

Iodometric titration of TBHP in heptane[11]**:** A sample of approximately 0.3 g of the TBHP solution was weighed accurately in a 500 mL Erlenmeyer and then 25 mL of a mixture of IPA and AcOH (96/4 v/v) and 2 mL of a saturated solution of KI in water were added. A white suspension was formed, which immediately turned yellow. A condenser was connected to the erlenmeyer and the erlenmeyer was then heated above a hotgun. Heating was only stopped when the mixture had refluxed for 30–60 seconds.

Immediately after this, 100 mL of water was added and the mixture was titrated with a 0.1 M $Na_2S_2O_4$-solution in water (for a 65 m% TBHP-solution, 40 to 45 mL of $Na_2S_2O_4$-solution is used).

Titration of aqueous H_2O_2 with $KMnO_4$: A sample of approximately 0.6 g of the H_2O_2 solution was weighed accurately in a 500 mL erlenmeyer flask and dissolved in 200 mL of water. This solution was titrated with a 0.05 M $KMnO_4$-solution in water (for a 35 M% H_2O_2 solution, about 50 mL of $KMnO_4$-solution is used).

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27. Harben, S.M.; Smith, P.D.; Helliwell, M.; Collison, D.; Garner, C. D., *J. Chem. Soc., Dalton Trans.*, **1997**, 4517–4523.
28. See crystal structure as discussed in Chapter 4.

VANADIUM-CATALYZED CYANIDE ADDITION TO ALDEHYDES

7

7.1. Introduction

Traditionally, catalytic asymmetric cyanohydrin synthesis has mostly been performed using enzymatic methods, peptide catalysis, or intramolecular chiral induction to direct the cyanation.[1] Since the last decade, there has been an explosion of interest in Lewis acid-catalyzed cyanation methods, which have proven to offer viable alternatives for the traditional methods.[2,3,4] One of the most successful Lewis acid catalytic systems investigated is based on the tetradentate salen ligand.[5,6] Whereas the investigations initially concentrated on the use of titanium complexes, the system appeared to perform better when vanadium was used.[7,8] This sparked the curiosity as to whether it is possible to use amavadin as a Lewis acid catalyst for (asymmetric) cyanohydrin synthesis. Catalytic activity of amavadin has only been observed in oxidation reactions (see Chapter 6) and the aim was to investigate whether the catalytic properties can be extended to the synthesis of cyanohydrins.

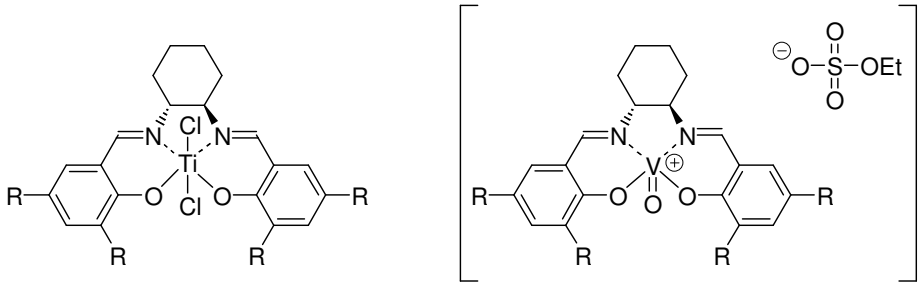
Before investigating the application of amavadin, we carried out experiments with the catalytic vanadium-salen system as reported in the literature.[8,9] The aim was not only to gain the expertise in this area of catalysis by reproducing the literature results, but also to induce a certain ligand modification of the system.

7.2. Vanadium-salen catalyzed asymmetric cyanohydrin synthesis

7.2.1. Introduction

After the initial reports in 1996 on titanium-salen catalyzed asymmetric cyanohydrin synthesis,[5,6] several optimizations of the system were performed by implementing changes in the ligand, the reaction conditions and the metal. The most effective catalysts that emerged from these investigations are summarized in Figure 7.1 as **1a**, **1b** and **2a**, the *ee*'s of the cyanation of benzaldehyde being given in brackets. It was reported in 1998 by the Belokon'/North partnership that an optimum performance was reached by using a cyclohexane ring in the backbone and *tert*-butyl substituents on the phenyl rings (complex **1a**).[10,11] In 2000, the same researchers achieved further improvement by using the *tert*-butyl substituted ligand with vanadium instead of titanium (complex **2a**).[7,8] In 2002, Liang and Bu reported that the enantioselectivity of the titanium system increased drastically by substitution of the *tert*-butyl groups for *tert*-amyl groups (complex **1b**).[12] The stereodirecting effect of the *tert*-amyl group is also manifested in Mn-salen epoxidation reactions.[13] Although the exact comparison of the performances of **1a**, **1b** and **2a** remains difficult due to small differences in the experimental set-up used by the researchers, the trends that are seen in the literature as expressed in Figure 7.1 are obvious. The figures pose the question as to how effective the combination of the *tert*-

amyl salen ligand and vanadium (i.e. complex **2b**) would be in asymmetric catalytic cyanohydrin synthesis. The aim was therefore to synthesize complex **2b**, to test it in the asymmetric cyanohydrin synthesis and to compare its performance with the performance of **1a**, **1b** and **2a**.



1a: R = *t*-Bu (88% *ee)* **2a**: R = *t*-Bu (94% *ee)*

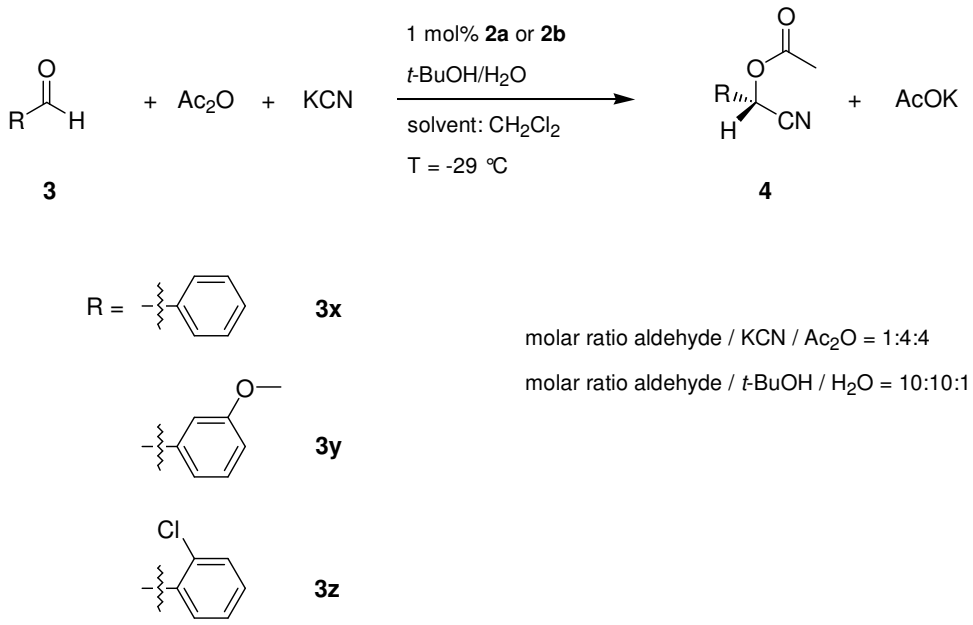
1b: R = *t*-Am (97% *ee)* **2b**: R = *t*-Am ?

Figure 7.1. *Optimized catalysts **1a**, **1b** and **2a**, with the ee's of benzaldehyde cyanohydrin given in brackets.*

7.2.2. Results and discussion

The synthesis of vanadium complex **2a** was performed according to the literature[8] procedure, starting with the synthesis of the ligand from 3,5-di-*tert*-butyl-salicylaldehyde and (*R*,*R*)-1,2-cyclohexanediamine. Reaction of the ligand with $VOSO_4$ gave **2a** in 47% yield (lit: 53%). The synthesis of **2b** was performed via the same method, although the 3,5-di-*tert*-amyl-salicaldehyde is not commercially available and needed to be prepared from 2,4-di-*tert*-amylphenol.[12] Reaction of the *tert*-amyl salen ligand with $VOSO_4$ gave complex **2b** in a yield of 49%.

The cyanation reactions were performed according to the literature procedure[9] using KCN as the cyanide source in the presence of acetic anhydride. The reaction set-up includes the use of *tert*-butanol and water as additives, which are reported to greatly accelerate the reaction. The reaction was run under a nitrogen atmosphere for 15 hours at –29 °C, after which the cyanohydrins were isolated and analyzed as their acetates (Scheme 7.1). The only difference with the literature procedure was the reaction temperature. For technical reasons, it was not possible to carry out the reaction overnight at –42 °C. However, the reported studies on the temperature dependence of the enantioselectivity show that this difference in temperature has a only marginal effect on the enantioselectivity.[9]



Scheme 7.1. *Catalytic asymmetric cyanohydrin syntheses using vanadium complexes **2a** and **2b**.*

The results of the catalytic cyanation reactions are shown in Table 7.1. The *ee*'s obtained with catalyst **2a** are in good agreement with the values reported in the literature and repetition of the experiments gave only small deviations in *ee*. The conversions are somewhat lower, and they suffered from deviations of 5–10% in different experiments. In the initial catalytic tests however, much lower conversions were obtained, and it appeared that grinding the KCN followed by drying in a desiccator prior to the reaction helped to solve that problem. This suggests that the low yields probably originated from a limited phase transfer.[14] This seems to be in conflict with the observed positive effect of the addition of water to the reaction, but it is reasonable to assume that adding the water at a later stage will have a different effect on the clustering of KCN and therefore on the phase transfer.

Table 7.1. *The results of the catalytic reactions[1)] using the vanadium catalysts **2a** and **2b**.*

substrate	catalyst 2a		catalyst 2b	
	conversion (%)[2)]	*ee* product (%)[2)]	conversion (%)	*ee* product (%)
3x	69 (87)	87 (90)	85	87
3y	88 (97)	95 (85)	82	90
3z	66 (99)	89 (77)	68	68

1) Selectivity is generally >98%, thus giving yields that equal the conversions.
2) The numbers in brackets represent the values reported in the literature

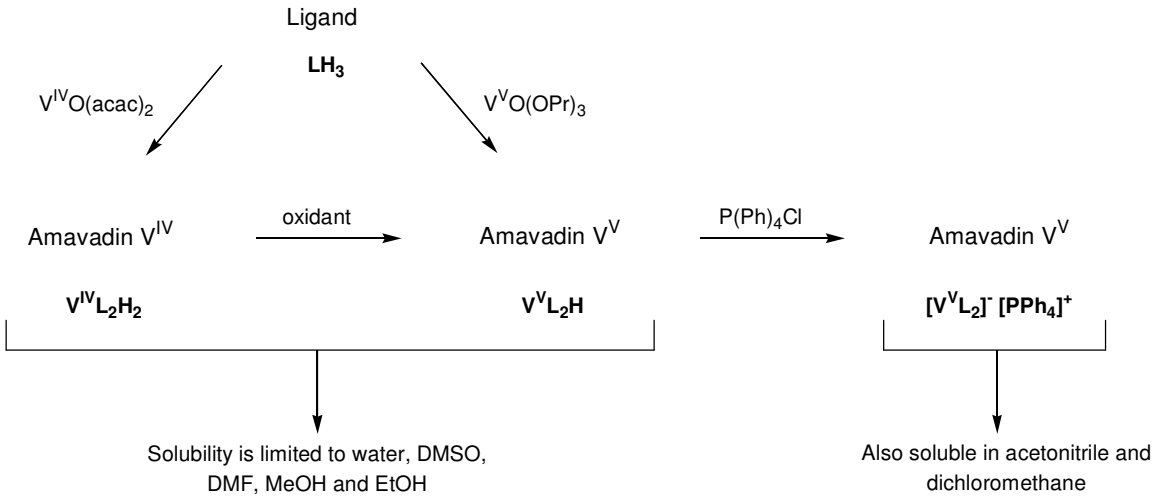
When comparing the *ee*'s obtained with vanadium complexes **2a** and **2b**, it can be concluded that the substitution of the *tert*-butyl with the *tert*-amyl substituents does not have the positive effect as observed for the titanium complexes. Substrate **3x** is converted with a similar

enantioselectivity by both catalysts, while **3y** and **3z** even gave lower *ee*'s when catalyst **2b** is used. Although the reaction mechanisms are believed to be the same for both metals,[7] the effect of the variation of the ligand is apparently different. In fact, the vanadium system has only been investigated using the *tert*-butyl substituted ligand, which is the ligand that gave (at the time) the best results on titanium. The results obtained with the *tert*-amyl ligand indicate that the trend of enantioselectivity versus ligand structure may be different with vanadium.

7.3. Application of amavadin in cyanohydrin synthesis

7.3.1. Introduction

The solubility profile of amavadin has been outlined in Chapter 6 and the scheme that summarizes the solubility behaviour of the different amavadin species is reproduced below (Scheme 7.2).



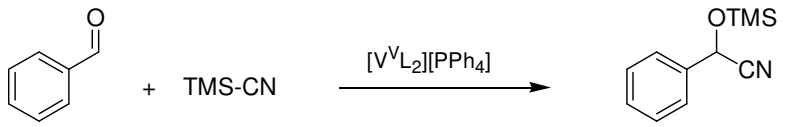
Scheme 7.2. *Overview of the solubility behaviour of the different amavadin species.*

It was reported in Chapter 6 that amavadin's solubility properties limit the application of amavadin as a homogeneous oxidation catalyst. For application in catalytic cyanide addition reactions, the consequences are similar. Again, the positive consequence of the high water-solubility of amavadin is the relatively easy separation of substrate and/or product from the catalyst. On the other hand, however, the lack of solubility in most organic solvents puts severe restrictions on the application in water-free media. The only way of obtaining a homogeneous amavadin solution in these media appeared to be use of $[V^{V}L_2][PPh_4]$, the oxidized amavadin with a PPh_4^+ counterion.

7.3.2. Results and discussion.

First, the catalytic activity was investigated in an aqueous-organic two-phase solvent system, which is a proven concept for the enzymatic synthesis of cyanohydrins.[15] In such a reaction system, the catalyst resides in the aqueous (working) phase and the reactants and products reside in the organic (extractive) phase. The pH is generally maintained low (e.g. pH 5) to reduce the rate of the non-catalyzed reaction. The amavadin V(IV) was tested using benzaldehyde as a substrate and toluene as the organic phase. Unfortunately, the product mandelonitrile could not be detected, not even after performing variations in the order of addition of substrate, the catalyst loading or the organic solvent (diisopropylether). When the oxidized amavadin compounds $V^{V}L_2H$ and $[V^{V}L_2][PPh_4]$ were applied under the same conditions, product formation could not be observed either. Moreover, the oxidized amavadin slowly reverted to the +4 oxidation state during the application of $V^{V}L_2H$, a behaviour that was also seen in the solution studies reported in Chapter 5. Application of $VO(acac)_2$ under the same conditions did not give any conversion either.

In a second approach, the $[V^{V}L_2][PPh_4]$ compound was applied under non-aqueous conditions using trimethylsilyl cyanide (TMS-CN) as the cyanating agent. This type of reaction is usually named a 'trimethylsilylcyanation', and generally has a low background reaction (Scheme 7.3).



Scheme 7.3. *Trimethylsilylcyanation of benzaldehyde leads to the mandelonitrile TMS-ether.*

The trimethylsilylcyanation reactions with $[V^{V}L_2][PPh_4]$ were performed at room temperature in dichloromethane, using benzaldehyde as the substrate in a set-up similar to the one reported by Belokon' *et al.*[11] Full conversion to racemic mandelonitrile TMS-ether was observed after 24 hours. However, when a catalytic amount of PPh_4Br or PPh_4Cl[16] was used instead of $[V^{V}L_2][PPh_4]$, full conversion to the TMS-ether was also reached after the same reaction time. These results indicate catalytic activity of the PPh_4^+-ion, which is supported by an earlier literature report on the catalytic action of phosphonium salts in the trimethylsilylcyanation of aldehydes and ketones.[17] A more realistic picture of the $[V^{V}L_2][PPh_4]$ species would therefore be to consider it as a Lewis acid – Lewis base pair, in which the $[PPh_4]^+$ is a Lewis acid catalyst and the $[V^{V}L_2]^-$ is (possibly) a Lewis base catalyst. Depending on the separation of the $[V^{V}L_2][PPh_4]$ ion pair and the reaction conditions, the environment of the $[PPh_4]^+$ ion can still be chiral, which could lead to stereoinduction in the catalytic reaction. It was investigated if a lower temperature could induce stereoinduction by performing the reaction at –10 °C, but this

appeared not to be the case. Then, the use of acetonitrile as a solvent was attempted, as it is one of the few aprotic solvents that is able to dissolve the $[V^{V}L_2][PPh_4]$ species. The rate in acetonitrile was similar to that in dichloromethane, and the resulting TMS-ether was also racemic. It is worth mentioning that a much faster background reaction was observed in acetonitrile. This is in accordance with an earlier report that describes a catalyst-free multicomponent Strecker reaction in acetonitrile.[18] This catalytic system also makes use of TMS-CN and the authors state that acetonitrile is by far the best solvent. An overview of the results obtained in the catalytic and non-catalytic cyanide addition experiments using the aqueous and non-aqueous experimental set-ups is given in Table 7.2.

Table 7.2. *Overview of the conversions and ee's obtained after 24 hours in the aqueous and non-aqueous catalytic cyanide addition reactions.*

Solvent	**Catalyst**							
	No catalyst		**Amavadin V(IV): VL_2H_2**		**Amavadin V(V): $[VL_2][PPh_4]$**		**PPh_4Br**	
	%conv.	%*ee*	%conv	%*ee*	%conv.	%*ee*	%conv	%*ee*
Water[1)]	0	–	0	–	0	–	n.a.	
Dichloromethane[2)]	0	–	n.a.		99	0	99	0
Acetonitrile[2)]	27	0	n.a.		99	0	99	0

1) The cyanide is added as KCN (from which HCN is formed in the reaction mixture)
2) The cyanide is added as TMS-CN

In conclusion, it can be stated that amavadin in its +4 oxidation state is not an active catalyst in the synthesis of cyanohydrins. On the other hand, the oxidized amavadin complex $[V^{V}L_2][PPh_4]$ is a good catalyst for the addition of TMS-CN to benzaldehyde, although the reaction is not stereoselective. It is not clear if the catalytic activity originates from the PPh_4-cation or from the vanadium(V)-anion, because blank experiments with $[PPh_4]^+[X]^-$ show that V is not required. The observed absence of stereoinduction in the reaction catalyzed by $[VL_2][PPh_4]$ can be a result of the presence of the vanadium complex as a mixture of two diastereomers. It is important to realize that non-linear effects can exist in such systems, leading to either asymmetric amplification or asymmetric depletion (see also the introduction of Chapter 6). It is, however, most likely that the observed lack of stereoinduction is due to the fact that the catalytic activity comes from the achiral PPh_4-cation.

It can furthermore be stated that the vanadium in amavadin is not a good Lewis acid in general, no matter if it exists in the +4 or the +5 oxidation state. This is due to the fact that amavadin bears two triprotic ligands, which makes the complex Brønsted acidic. After deprotonation or after substitution of a proton for PPh_4^+, the negatively charged vanadium species has poor Lewis acid properties.

7.4. Experimental

General considerations. Column chromatography was carried out with silicagel 0.060-0.200 mm, pore diameter approx. 6 nm. TLC was performed on 0.20 mm silica gel and developed in a ninhydrin bath (15 g of vanillin in 250 ml ethanol + 10 ml of conc. H_2SO_4), where the products containing a CN group gave orange spots after heating the plate. HPLC-analysis was performed on a 4.6 x 250 mm 10 µm Chiracel OD column, using eluates consisting of isopropanol in hexane in a flow of 0.8 ml min^{-1}. The retention times and the eluent compositions for products **4x**, **4y** and **4z** are shown in Table 7.3.

Table 7.3. *The retention times and the eluent compositions for products **4x**, **4y** and **4z**.*

Product	Eluent	Retention time (min)	
	(% *i*-PrOH in hexane)	(*R*)-enantiomer	(*S*)-enantiomer
4x	2	12.8	14.2
4y	10	9.9	11.8
4z	2	10.6	12.8

The *tert*-butyl salen ligand [=(1*R*,2*R*)-*N*,*N*'-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine] and the corresponding vanadium complex **2a** were prepared according to the literature procedure.[8] The vanadium-salen catalyzed asymmetric synthesis of the acetate protected cyanohydrins **4a**, **4b** and **4c** was performed according to the literature procedure.[9] The aldehydes for the cyanations were distilled prior to use, potassium cyanide was dried in a desiccator prior to use and other chemicals were used as received. Acetic anhydride was obtained from Baker, trimethylsilyl cyanide was obtained from Acros.

Synthesis of 3,5-di-*tert*-amyl-2-hydroxybenzaldehyde. The synthesis was performed according to the literature procedure[12] with some modifications. A mixture of 2.91 g of 2,4-di-*tert*-amylphenol (12.4 mmol), 3.41 g of hexamethylenetetramine (24.3 mmol) and 30 ml of acetic acid (0.5 mol) was heated over a period of 30 minutes to 130 °C (reflux temperature). After 2 hours of reflux, the temperature was reduced to 75 °C and 30 ml of sulfuric acid (30% (w/w)) was added, after which a turbid light-orange mixture formed. This mixture was stirred for another 90 minutes at 110 °C, the temperature was then reduced to 75 °C and 25 mL of petroleum ether was added. The organic layer was separated and the water layer was washed with 2 x 25 ml of petrolether. The combined layers of petrol ether were evaporated and purified over a plug of silica, using a 4:1 ratio of petrol ether and ethyl acetate. The product was isolated in 2.31 g (8.80 mmol, 71%) of a yellow liquid. ^{1}H NMR ($CDCl_3$) δ; 11.6 (s, 1H, ArO<u>H</u>), 9.9 (s, 1H, C<u>H</u>O), 7.4 (d, *J*= 2.4, 1H, C<u>H</u>$_{Ar}$), 7.3 (d, *J*= 2.4, 1H, C<u>H</u>$_{Ar}$), 1.89 (q, *J*= 7.5, 2H, C<u>H</u>$_2$CH$_3$), 1.63 (q, *J*= 7.5, 2H, C<u>H</u>$_2$), 1.38 (s, 6H, C(C<u>H</u>$_3$)$_2$), 1.28 (s, 6H, C(C<u>H</u>$_3$)$_2$), 0.68 (t, *J*= 7.5, 3H, CH$_2$C<u>H</u>$_3$). ^{13}C NMR ($CDCl_3$) δ; 197.4 (<u>C</u>HO), 159.0 (<u>C</u>$_{Ar}$CHO), 139.7 (<u>C</u>$_{Ar}$C$_{t\text{-Am}}$), 135.8 (<u>C</u>$_{Ar}$C$_{t\text{-Am}}$), 133.8 (H<u>C</u>$_{Ar}$), 128.7 (H<u>C</u>$_{Ar}$), 119.9 (<u>C</u>$_{Ar}$OH), 38.6 (<u>C</u>H$_2$CH$_3$), 37.4 (<u>C</u>H$_2$CH$_3$), 36.7 (<u>C</u>(CH$_3$)$_2$(C$_2$H$_5$)), 32.6 (<u>C</u>(CH$_3$)$_2$(C$_2$H$_5$)), 28.4 (2C, C<u>C</u>H$_3$), 27.4 (2C, C<u>C</u>H$_3$), 9.4 (CH$_2$<u>C</u>H$_3$), 9.1 (CH$_2$<u>C</u>H$_3$).

Synthesis of the *tert*-amyl salen ligand [=(1*R*,2*R*)-*N*,*N*'-bis(3,5-di-*tert*-amylsalicylidene)-1,2-cyclohexanediamine]. The protocol that was used for the synthesis of the *tert*-amyl salen ligand is based on the procedure reported[8] for the *tert*-butyl ligand. In a 250 mL three-necked flask, 234 mg (2.05 mmol) of (*R*,*R*)-1,2-cyclohexanediamine was dissolved in a mixture of 10 mL of water and 40 mL of ethanol. This mixture was heated to reflux and then a solution of 1.181 g (4.5 mmol) of 3,5-di-*tert*-amylsalicylaldehyde was added during two minutes. The yellow slurry that was formed was stirred under reflux for three hours. After the addition of 10 mL of water, the mixture was crystallized overnight at 4 °C. After filtration, the crude product was dissolved in 45 mL of CH_2Cl_2 and was washed with 2 x 25 mL of water and 1 x 10 mL of brine. Drying overnight on Na_2SO_4 and recrystallization from ethanol yielded 996 mg (1.65 mmol, 81%) of a yellow solid. Mp = 146 °C. ^{1}H NMR ($CDCl_3$) δ; 13.6 (s br, 2H, ArO<u>H</u>), 8.3 (s, 2H, C<u>H</u>=N), 7.2 (s, 2H, C<u>H</u>$_{Ar}$), 6.9 (s, 2H, C<u>H</u>$_{Ar}$), 3.3 (s, 2H, <u>H</u>C-N), 2.0-1.4 (m, 8H, 4 x C<u>H</u>$_2$), 1.53 (q, *J*= 7.3, 8H, 4 x C<u>H</u>$_2$), 1.34 (s, 12H, 2 x C(C<u>H</u>$_3$)$_2$), 1.19 (s, 12H, 2 x C(C<u>H</u>$_3$)$_2$), 0.62 (t, *J*= 7.4, 6H, (CH$_2$C<u>H</u>$_3$)$_2$), 0.59 (t, *J*= 7.5, 6H, (CH$_2$C<u>H</u>$_3$)$_2$). ^{13}C NMR ($CDCl_3$) δ; 166.2 (2C, H<u>C</u>=N), 158.0 (2C, <u>C</u>$_{Ar}$OH),138.2 (2C, <u>C</u>$_{Ar}$), 134.8 (2C, <u>C</u>$_{Ar}$), 128.9 (2C, H<u>C</u>$_{Ar}$), 126.8 (2C, H<u>C</u>$_{Ar}$), 117.6 (2C, <u>C</u>$_{Ar}$), 72.1 (2C, H<u>C</u>-N), 38.5 (2C, H$_2$<u>C</u>CN), 37.2 (2C, <u>C</u>CH$_2$), 36.8 (2C, <u>C</u>CH$_2$), 33.0 (2C, C<u>C</u>H$_2$), 32.7 (2C, C<u>C</u>H$_2$), 28.3 (4C, C<u>C</u>H$_3$), 27.4 (4C, C<u>C</u>H$_3$), 24.3 (2C, <u>C</u>H$_2$<u>C</u>H$_2$), 9.5 (2C, H$_2$C<u>C</u>H$_3$), 9.1 (2C, H$_2$C<u>C</u>H$_3$). $[\alpha]_D^{20}$ = –316° (c=1, CH_2Cl_2).

Synthesis of vanadium-salen complex 2b. In a 250 mL three-necked flask equipped with a thermometer and a cooler, 211 mg (0.972 mmol) of $VOSO_4 \cdot 3H_2O$ was added to 35 mL of ethanol. At reflux temperature, most of the $VOSO_4$ was dissolved. During one minute, a solution of 574 mg (0.952 mmol) of the ligand [=(1*R*,2*R*)-*N*,*N*'-bis(3,5-di-*tert*-amylsalicylidene)-1,2-cyclohexanediamine] in 20 mL of THF was added, after which the colour immediately turned dark green. This mixture was heated under reflux for three hours, after which the solvents were removed in vacuo. The residue was dissolved in dichloromethane and filtered. The filtrate was evaporated to dryness and the crude product was purified over a column of silica, eluting first with dichloromethane and then with ethyl acetate / methanol 2 / 1). Complex **2b** was obtained as a green product in a yield of 0.376 g (0.464 mmol, 49%). ^{1}H NMR ($CDCl_3$) δ; 8.8 (s, 1H, C<u>H</u>=N), 8.53 (s, 1H, C<u>H</u>=N), 7.63 (d, *J*= 2.1, 1H, C<u>H</u>$_{Ar}$), 7.58 (d, *J*= 2.1, 1H, C<u>H</u>$_{Ar}$), 7.50 (d, *J*= 1.8, 1H, C<u>H</u>$_{Ar}$), 7.44 (d, *J*= 1.8, 1H, C<u>H</u>$_{Ar}$), 4.30 (m, 1H, <u>H</u>C-N) 3.78 (m, 1H, <u>H</u>C-N), 3.44 (m, 2H, SO<u>H</u>$_2$CCH$_3$), 1.8-2.8 (m, 8H, 4 x C<u>H</u>$_2$), 1.7 (q, 4H, 2 x <u>H</u>$_2$CCH$_3$), 1.6 (q, 4H, 2 x <u>H</u>$_2$CCH$_3$), 1.5 (s, 12H, 2 x C(C<u>H</u>$_3$)$_2$), 1.4 (s, 12H, 2 x C(C<u>H</u>$_3$)$_2$), 0.83 (t, 6H, 2 x CH$_2$(C<u>H</u>$_3$)), 0.70 (t, 6H, 2 x CH$_2$(C<u>H</u>$_3$)), 0.6 (t, 3H, SOCH$_2$C<u>H</u>$_3$). ^{13}C NMR ($CDCl_3$) δ; 164.7 (2C, <u>C</u>-OV), 161.1 (H<u>C</u>=N), 161.0 (H<u>C</u>=N), 142.2 (<u>C</u>$_{Ar}$), 142.1 (<u>C</u>$_{Ar}$), 134.3 (<u>C</u>$_{Ar}$), 133.8 (<u>C</u>$_{Ar}$), 133.4 (H<u>C</u>$_{Ar}$), 133.3 (H<u>C</u>$_{Ar}$), 130.1 (H<u>C</u>$_{Ar}$), 129.2 (H<u>C</u>$_{Ar}$), 121.9 (<u>C</u>$_{Ar}$), 120.8 (<u>C</u>$_{Ar}$), 70.7 (H<u>C</u>-N), 70.0 (H<u>C</u>-N), 63.1 (SO<u>C</u>H$_2$), 39.3 (H$_2$<u>C</u>CN), 39.1 (H$_2$<u>C</u>CN), 37.7 (<u>C</u>CH$_2$), 37.6 (<u>C</u>CH$_2$), 36.9 (<u>C</u>CH$_2$), 36.8 (<u>C</u>CH$_2$), 33.5 (C<u>C</u>H$_2$), 33.3 (C<u>C</u>H$_2$), 30.8 (C<u>C</u>H$_2$), 29.2 (C<u>C</u>H$_2$), 28.6 (C<u>C</u>H$_3$), 28.5 (C<u>C</u>H$_3$), 28.4 (C<u>C</u>H$_3$), 28.3 (C<u>C</u>H$_3$), 27.8 (C<u>C</u>H$_3$), 27.7 (C<u>C</u>H$_3$), 27.6 (C<u>C</u>H$_3$), 27.5 (C<u>C</u>H$_3$), 24.5 (<u>C</u>H$_2$CH$_2$), 24.1 (<u>C</u>H$_2$CH$_2$), 14.7 (SOCH$_2$<u>C</u>H$_3$), 9.5 (H$_2$C<u>C</u>H$_3$), 9.4 (H$_2$C<u>C</u>H$_3$), 9.2 (H$_2$C<u>C</u>H$_3$), 9.1 (H$_2$C<u>C</u>H$_3$). $[\alpha]_D^{20}$ = –1400 ° (c=0.01, $CHCl_3$). MS (*m*/*z*) 668 (ES^+, M – H_2O – $[EtOSO_3]^-$).

Attempts to perform amavadin catalyzed hydrocyanations in water. The procedure was based on the literature with some modifications.[15] In a closed flask, a solution of 130 mg (2 mmol) of KCN and 0.02 mmol of the appropriate amavadin compound (i.e. $V^{IV}L_2H_2$, $V^{V}L_2H$ or $[V^{V}L_2][PPh_4]$) in 3 mL of water was adjusted to pH 5 by the addition of 1 M HCl solution. A solution of 106 mg (1 mmol) of benzaldehyde in 3 mL of toluene was added to the aqueous catalyst solution, after which the reaction mixture was monitored in time. Samples were taken after 30 minutes, 7 hours and 24 hours and analyzed with TLC and/or HPLC.

Amavadin-catalyzed trimethylsilylcyanation of benzaldehyde. The procedure was based on the literature with some modifications.[11] A solution of 10 mg (0.013 mmol) of the $[VL_2][PPh_4]$ complex in 1.5 mL of solvent (dichloromethane or acetonitrile) was prepared in a 20 mL schlenk flask under nitrogen. Then 0.130 mL (1.28 mmol) of benzaldehyde and 0.380 mL (2.85 mmol) of TMS-CN were added to the catalyst solution, after which the reaction mixture was monitored in time. Samples were taken after 30 minutes, 7 hours and 24 hours, purified over a 1 cm silica plug with dichloromethane as the eluent, and analyzed with chiral GC.

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Summary

In 1931, the TU Delft professor Ter Meulen discovered vanadium accumulation in *Amanita muscaria* (fly agaric), which marked the start of amavadin as a subject of scientific research. The research described in this thesis is focused on the synthesis and structure of amavadin and related complexes. Secondly, explorations of amavadin's catalytic properties are reported.

Chapter 1 provides a general introduction to the chemistry of vanadium. The areas of vanadium involvement in biological systems are classified and described, which puts amavadin into the context of biosynthesized vanadium complexes. Secondly, the basis of vanadium's ability to act as a Lewis acid catalyst is given, followed by an overview of the application of vanadium in homogeneous catalysis, highlighting asymmetric reactions. Finally, an introduction to amavadin is given in a scientific as well as a historical context.

Chapter 2 describes the first enantioselective synthesis of the amavadin ligand and the preparation of amavadin. The basis of the enantioselective route is the use of triflate leaving groups, which effects the desired complete inversion of configuration during the nucleophilic substitutions of hydroxylamine. After the preparation of amavadin and its optical antipode, the epimerization of both complexes was followed using polarimetry and NMR.

The aim of Chapter 3 was to synthesize amavadin-like ligands, the relationship with the amavadin ligand being that these ligands are also *N*-substituted *N*-hydroxy amino acids. After the recognition that the traditional nucleophilic substitution route is of limited applicability, a switch was made to nitrones as building blocks. The first approach comprised the addition of cyanide to nitrones, but the hydrolysis of the resulting α-cyano secondary hydroxylamines was unsuccessful. The second approach was based on nitrone reduction ('reductive hydroxylamination') and proved to be a versatile method to prepare the amavadin-based ligands. An interesting spin-off of the synthetic work on nitrones is an effective and easily applicable method to obtain racemic *N*-hydroxy amino acid esters.

Chapter 4 is concerned with the main goal of this thesis: to establish which factors are important for the formation of 'amavadin-type complexes' and to further explore the structural properties of such complexes. The ligands that are described in Chapter 3 were therefore complexed with vanadium. By using the *meso*-ligand of amavadin, the synthesis and X-ray crystallographic study of '*meso*-amavadin' was performed. Based on NMR- and X-ray data of amavadin and '*meso*-amavadin', a model was developed that could be used to predict the ^{1}H and ^{13}C NMR spectra of amavadin analogues. This was demonstrated for two examples that contained a benzyl or a phenyl group at the ligand backbone. These two analogues furthermore

showed that increasing the size of the backbone substituents still gave vanadium(IV) non-oxo complexes that are analogous to amavadin. In contrast, protection of one carboxylate per ligand did not give a stable vanadium(IV) non-oxo compound, while complete lack of one carboxylic acid group resulted in immediate oxidation of the vanadium.

Chapter 5 discusses the physical and chemical properties of amavadin. These include its hydration/dehydration, its solubility and the observed autoreduction of oxidized amavadin in water. Based on this, the reactivity of amavadin with peroxides was studied, with the aim to get insight into the nature of the vanadium species present in oxidation catalysis. The addition of several equivalents of hydrogen peroxide to amavadin in water led to the dissociation of one ligand, and a large excess of hydrogen peroxide resulted in the loss of both ligands under the formation of the oxodiperoxovanadium(V) anion. In the case of *tert*-butyl hydroperoxide as the oxidant in water, similar processes took place.

Chapter 6 deals with the application of amavadin as an oxidation catalyst. It was shown that amavadin in water can act as an alcohol oxidation catalyst as well as a sulfoxidation catalyst: the oxidation of thioanisole yielded the sulfoxide and significant amounts of the sulfone. Better results were obtained for oxidations in non-aqueous and aprotic environments, using thoroughly dried amavadin in the absence of solvent. When *tert*-butyl hydroperoxide was used as the oxidant, epoxidation of cinnamyl alcohol could be achieved with high conversion and high yield. Similarly, the oxidation of benzyl alcohol led almost exclusively to benzoic acid, while no activity was observed in the oxidation of aliphatic alcohols. Despite the fact that amavadin is chiral, none of the oxidations occurred stereoselectively.

Chapter 7 is concerned with vanadium catalysts for cyanide addition reactions to aldehydes. Firstly, the catalytic vanadium-salen system as reported in the literature was modified: the *tert*-butyl groups in the ligand were substituted for *tert*-amyl groups. No increase of catalytic performance in terms of activity and enantioselectivity was observed in this case. Secondly, amavadin was tested as a catalyst for the cyanide addition reactions. It appeared that amavadin in its +4 oxidation state is not an active catalyst, but oxidized amavadin proved to be active indeed. In the discussion, a possible explanation is put forward that the catalytic activity originates from both the PPh_4-cation as well as the vanadium(V)-anion. Also, no stereoselectivity was observed.

Samenvatting

In 1931 ontdekte professor Ter Meulen aan de TU Delft dat zich in *Amanita muscaria* (vliegenzwam) vanadium ophoopt. Deze ontdekking kan worden beschouwd als de start van amavadin als onderwerp van wetenschappelijk onderzoek. Het in dit proefschrift beschreven onderzoek richtte zich op de synthese en de structuur van amavadin en gerelateerde complexen. In tweede instantie werden studies verricht naar de katalytische activiteit van amavadin.

Hoofdstuk 1 geeft een algemene introductie over de chemie van vanadium. De verschillende verschijningsvormen van vanadium in de natuur worden geclassificeerd en beschreven. Ten tweede wordt de activiteit van vanadium als Lewiszure katalysator beschreven, gevolgd door een overzicht van de toepassing van vanadium in homogene katalyse. Hierbij ligt de nadruk op asymmetrische omzettingen. Tot slot wordt een overzicht van amavadin gegeven in zowel een wetenschappelijk als een historisch kader.

Hoofdstuk 2 beschrijft de eerste enantioselectieve synthese van het amavadin ligand en de synthese van amavadin zelf. De essentie van de enantioselective route is het gebruik van triflaat als vertrekkende groep, hetgeen de vereiste complete inversie van configuratie bij de nucleofiele substitutie van hydroxylamine bewerkstelligt. Na de synthese van amavadin en zijn optisch tegengestelde complex werd de epimerisatie van beide complexen gevolgd met behulp van polarimetrie en NMR.

Het doel van Hoofdstuk 3 was om amavadin-gerelateerde liganden te synthetiseren, waarbij de relatie met amavadin bestaat uit het feit dat het allemaal *N*-gesubstitueerde *N*-hydroxy aminozure esters zijn. Nadat bleek dat de conventionele route van nucleofiele substitutie een beperkte toepasbaarheid heeft, is er voor een route gekozen die gebaseerd is op het gebruik van nitronen als bouwstenen. De eerste aanpak hierbij was de additie van cyanide aan nitronen, maar de hydrolyse van de daaruit voortgekomen α-cyano secundaire hydroxylamines was zonder succes. De tweede aanpak was gebaseerd op de reductie van nitronen (‘reductieve hydroxylaminering’) en dit bleek een veelzijdige methode om de amavadin-gerelateerde liganden te synthetiseren. Een interessante spin-off van het werk aan nitronen is een effectieve en makkelijk toepasbare methode om racemische *N*-hydroxy aminozure esters te maken.

Hoofdstuk 4 is gewijd aan het hoofddoel van dit proefschrift: vaststellen welke factoren belangrijk zijn voor het ontstaan van complexen van het amavadin-type en het verder bestuderen van de structuureigenschappen van zulke complexen. Hiertoe werden de liganden die beschreven zijn in Hoofdstuk 3 gecomplexeerd met vanadium. Met het *meso*-ligand van amavadin werd de synthese en de kristallografische studie van ‘*meso*-amavadin’ uitgevoerd.

Op basis van de NMR- en kristallografische data van amavadin en '*meso*-amavadin' werd een model ontwikkeld dat gebruikt kon worden om de ^{1}H and ^{13}C NMR spectra van amavadin-analoga te voorspellen. De werking hiervan werd aangetoond voor twee voorbeelden waarbij er op het ligandskelet een benzyl- of een fenylgroep zat. Deze analoga lieten verder zien dat het vergroten van de omvang van de groepen op het ligandskelet nog steeds leidt tot vanadium(IV) non-oxocomplexen analoog aan amavadin. Het beschermen van één carboxylgroep per ligand gaf daarentegen geen stabiel vanadium(IV) non-oxocomplex, terwijl de volledige afwezigheid van één van de carboxylgroepen een onmiddellijke oxidatie van het vanadium tot gevolg had.

Hoofdstuk 5 beschrijft de fysische en chemische eigenschappen van amavadin. Dit betreft de hydratie/dehydratie, de oplosbaarheid en de waargenomen autoreductie van amavadin in water. Op basis hiervan werd de reactiviteit van amavadin met peroxides bestudeerd, met het doel om meer inzicht te krijgen in de structuren van de vanadium-intermediairen die aanwezig zijn gedurende de oxidatiekatalyse. Het toevoegen van meerdere equivalenten waterstofperoxide aan amavadin in water leidde tot de dissociatie van één ligand, en een grote overmaat waterstofperoxide resulteerde in het verlies van beide liganden onder de vorming van het oxodiperoxovanadium(V) anion. In het geval van *tert*-butylhydroperoxide als oxidant in water vonden vergelijkbare processen plaats.

Hoofdstuk 6 gaat over de toepassing van amavadin als oxidatiekatalysator. Er werd aangetoond dat amavadin in water katalytisch actief is in zowel de oxidatie van alcoholen als van sulfides; de oxidatie van thioanisool gaf het sulfoxide en significante hoeveelheden van het sulfon. Betere resultaten werden behaald met oxidaties in watervrij en aprotisch medium. Hierbij werd goed gedroogde amavadin gebruikt in de afwezigheid van oplosmiddel. Wanneer *tert*-butylhydroperoxide werd gebruikt als oxidant, kon kaneelalcohol worden geoxideerd tot het epoxide met hoge conversie en hoge opbrengst. De oxidatie van benzylalcohol leidde bijna uitsluitend tot benzoëzuur terwijl geen activiteit werd gevonden voor de oxidatie van alifatische alcoholen. Ondanks het feit dat amavadin een chirale katalysator is, kon er geen stereoselectie tijdens oxidatie worden waargenomen.

Hoofdstuk 7 is gewijd aan vanadiumkatalysatoren voor cyanide-addities aan aldehydes. Als eerste werd het in de literatuur beschreven vanadium-salen systeem gemodificeerd: de *tert*-butyl groepen in het ligand werden vervangen door *tert*-amyl groepen. Er werd hier geen toename in de katalytische activiteit en enantioselectiviteit waargenomen. Ten tweede werd amavadin getest als katalysator voor de cyanide-addities. In de +4 oxidatietoestand bleek amavadin geen actieve katalysator, maar geoxideerde amavadin wel. In de discussie wordt aangevoerd dat deze katalytische activiteit zowel van het PPh_4-kation als van het vanadium(V)-anion afkomstig kan zijn. Ook in deze reacties werd geen enantioselectiviteit waargenomen.

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Curriculum vitae

Ton Hubregtse werd op 20 januari 1976 geboren te Winschoten. Hij behaalde in 1994 zijn Gymnasium diploma aan het Strabrecht College te Geldrop en in de zomer van datzelfde jaar begon hij met de studie Scheikunde aan de Rijksuniversiteit Groningen. In 1997 koos hij voor de afstudeerrichting 'Organometaalchemie & Homogene Katalyse' in de groep van Prof. dr. J. H. Teuben, waar hij de synthese en reactiviteit van vanadiumcomplexen bestudeerde. Gedurende het tweede deel van zijn afstudeerperiode verrichtte hij op het 'Shell Research and Technology Centre' in Amsterdam onderzoek naar de oligomerisatie van etheen. In 2000 werd het doctoraalexamen in de scheikunde (met genoegen) afgelegd. In 2001 trad hij in dienst van de Technische Universiteit Delft, waar hij zijn promotieonderzoek verrichtte op het gebied van amavadin. Dit was een gezamenlijk project van de secties 'Biokatalyse en Organische Chemie' o.l.v. Prof. dr. R. A. Sheldon en 'Toegepaste Organische Chemie en Katalyse' o.l.v. Prof. dr. Th. Maschmeyer. De resultaten hiervan zijn in dit proefschrift beschreven.