COMBINATORIAL APPROACHES TOWARDS

ACTIVE MODELS FOR GALACTOSE OXIDASE

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Magali Dousset

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COMBINATORIAL APPROACHES TOWARDS

ACTIVE MODELS FOR GALACTOSE OXIDASE

Berichterstatter: Prof. Dr. A. Berkessel

                                                 Prof. Dr. H.-G. Schmalz

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"Das Leben ist zu kostbar, um es dem Schicksal zu überlassen."

Walter Moers

Die 13$^{1/2}$ Leben des Käpt'n Blaubär

Acknowledgement

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1   Summary

This work deals with the synthesis of copper complexes as models for the enzyme galactose oxidase. The latter were tested in the catalytic aerobic oxidation of primary alcohols.

The first models for the active site of galactose oxidase are the libraries of 81 decapeptide–copper complexes represented in **Figure 1-1**.
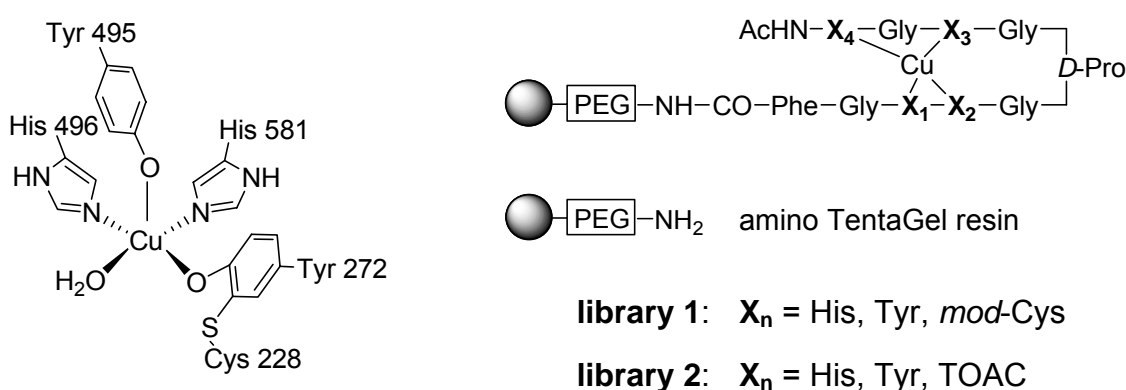
Figure 1-1:   **Active site of GOase and libraries of peptide–copper complexes designed to mimic the active site of the enzyme.**

The decapeptides contain four variable positions occupied in a combinatorial fashion by histidine, tyrosine or the unnatural amino acids TOAC **41** or *mod*-Cys **42**. Histidine and tyrosine were chosen because of their presence at the active site of the enzyme, while TOAC and *mod*-Cys should mimic the Tyr 272–Cys 228 conjugate and act as a redox centre.
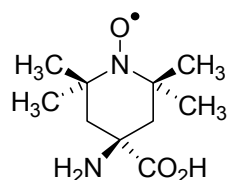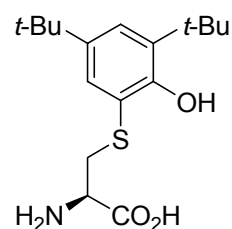
TOAC, **41**   *mod*-Cys, **42**

The modified cysteine *mod*-Cys was synthesised with Boc- and Cbz-protected amino function (**47** and **53**), while the *N*-Fmoc-protected *mod*-Cys could not be obtained.
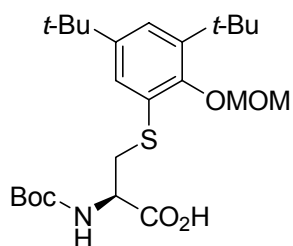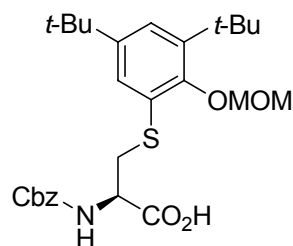
47   **53**

The two *mod*-Cys containing decapeptides **82** and **83** were synthesised on amino TentaGel resin in order to show that it was possible to incorporate the *N*-Boc protected *mod*-Cys building block **47** in a peptide sequence.

	X_1 = *mod*-Cys	X_1 = *mod*-Cys
	X_2 = Tyr	X_2 = His
	X_3 = His	X_3 = His
	X_4 = His	X_4 = Tyr
	82	**83**
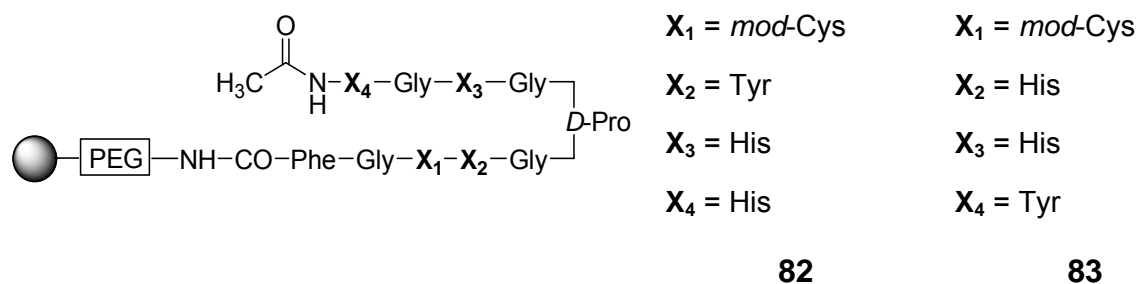
The two decapeptides were incubated in a copper(II) salt solution and the resulting peptide–copper complexes were used as catalysts in the aerobic oxidation of 3-methoxybenzyl alcohol. A turnover number of 40 was observed after 24 hours with the copper complex of peptide **82** in neat alcohol and potassium hydroxide as a base.

The 81-mer library including TOAC (**Figure 1-1**, library 2) was synthesised following the split-mix protocol and the *IRORI*-"directed synthesis" system. The decapeptides were synthesised on amino TentaGel resin. After acetylation of the *N*-terminus, the solid-supported peptide-ligands were incubated in a copper(II) acetate solution. The observation of the resulting resin beads under the microscope revealed a particular colour for each peptide sequence. These different colours demonstrate the affinity of the copper ion for the functional groups of the peptide-ligands.

Figure 1-2: Microscope view of the solid-supported peptide–copper complexes.

The peptide-ligands were screened for activity in the copper-catalysed aerobic oxidation of primary alcohols. 3-Methoxybenzyl alcohol was used as a test substrate since the formation of the corresponding aldehyde can be followed by UV-spectroscopy.

absorption at λ = 310 nm

39   **40**
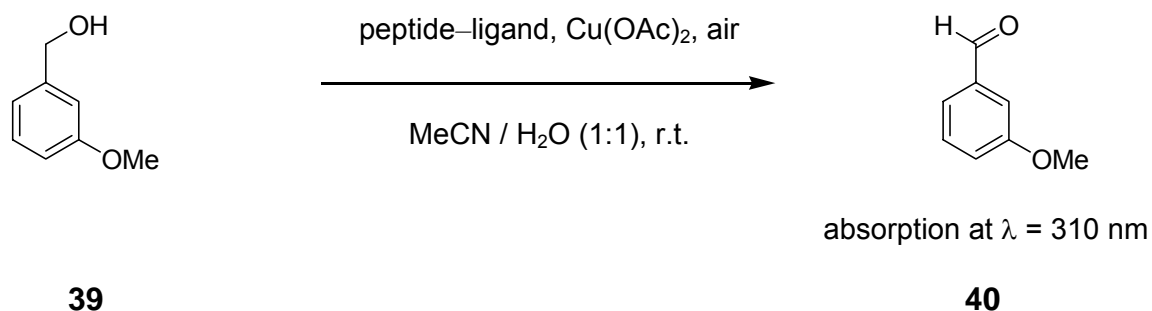
Scheme 1-1:   **Assay for the screening of the library of peptide–ligands.**

The screening allowed to distinguish between the members of the library and revealed some particularly interesting peptide sequences (**Figure 1-3**). A turnover frequency of $10^{-4}\,s^{-1}$ was obtained with the peptide ligand containing 2 tyrosine residues at the X_1 and X_2 positions and two TOAC residues at the X_3 and X_4 positions.
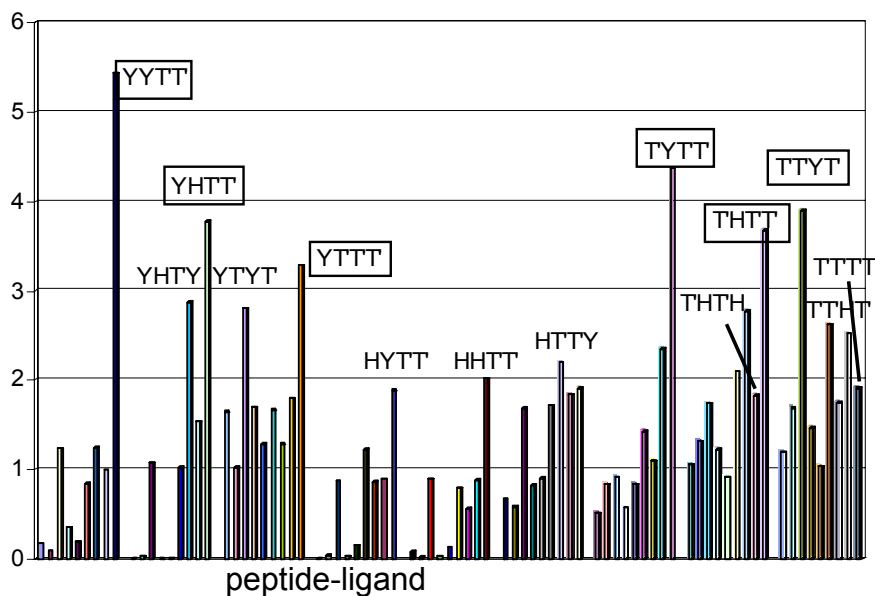
Figure 1-3: Screening of the 81-mer library of decapeptides 99. From left to right are all the members of the library in the order given in the experimental section with compound 99-01 to the left and compound 99-81 to the right. As an example, YYT'T' corresponds to the peptide-ligands where X_1 = Tyr, X_2 = Tyr, X_3 = TOAC and X_4 = TOAC.
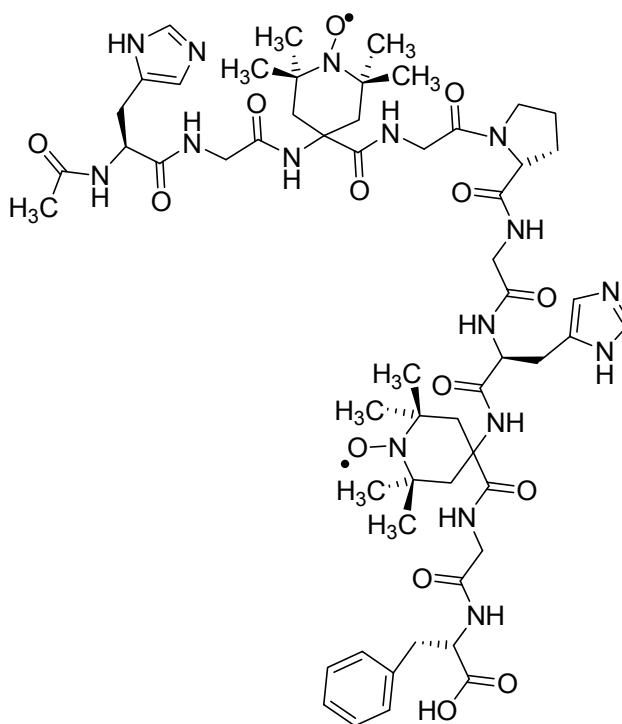
101

One of the peptide-ligands was synthesised on cleavable resin and subsequently cleaved from the resin. The activity of this peptide-ligand **101** proved to be higher than its solid-supported counterpart since 14.3 μmol of aldehyde were formed with the soluble copper complex and 7.29 μmol were formed with the solid-supported copper complex after 24 hours.

In another part of this work, the low molecular weight ligand **66** was synthesised on solid support. The in situ formed copper complex proved to be active in the catalytic aerobic oxidation of benzyl alcohol and 3-methoxybenzyl alcohol. A turnover frequency of $3 \cdot 10^{-3}$ s^{-1} was achieved with this solid-supported ligand.
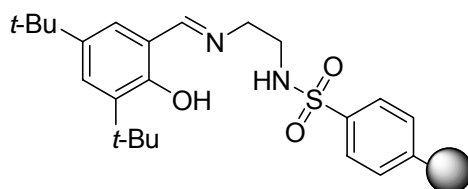
66

2   Introduction

Aldehydes constitute a group of very interesting compounds in organic chemistry. They occur as natural flavouring constituents in a wide variety of foods, often in relatively small, but occasionally in very large concentrations, and are therefore widely used as food additives.[1] Aldehydes also belong to the most frequently used intermediates in organic synthesis since they are relatively reactive compounds. The controlled oxidation of primary alcohols is a common way for the preparation of aldehydes. Traditionally, the oxidation of primary alcohols to aldehydes is performed with stoichiometric amounts of inorganic oxidants, notably chromium(VI)[2] (e.g. pyridinium chlorochromate in the *Corey* oxidation)[3] or with activated dimethylsulfoxide[4] (e.g. dimethylsulfoxide and dicyclohexylcarbodiimide in the *Pfitzner-Moffatt* oxidation,[5] or dimethylsulfoxide and oxalylchloride in the *Swern* oxidation).[6] More recently, catalysts containing heavy metals such as palladium[7] or ruthenium[8] have been developed. But these oxidising agents and catalysts are hazardous, toxic, relatively expensive or generate a lot of waste. Therefore, from both economical and environmental points of view, there is a demand for more effective methods that employ clean oxidants. Particularly, the use of dioxygen as a stoichiometric oxidant together with a cheap metal catalyst appears very attractive. A few synthetic catalysts using copper and molecular oxygen or air have been reported so far, notably by *Sheldon*,[9] *Semmelhack*[10] and *Markó*.[11, 12]

Copper is an abundant metal on earth and is found in various metalloproteins,[13] especially in enzymes involved in the binding of molecular oxygen.[14] An interesting example is found in the enzyme galactose oxidase (GOase) which catalyses the selective oxidation of primary alcohols to aldehydes, coupled with the reduction of dioxygen to hydrogen peroxide (**Figure 2-1**).[15]
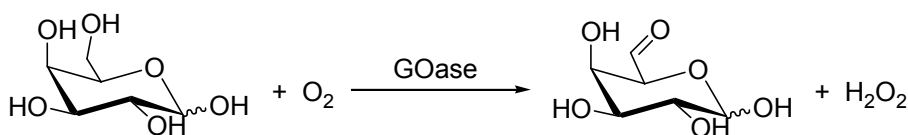
Figure 2-1: Oxidation of galactose by galactose oxidase.[15]

In the last fifteen years, various groups have been working on the synthesis of models for the enzyme galactose oxidase. Biomimetic low molecular weight copper

complexes have so far been synthesised by various groups, notably by *Stack*,[16, 17] *Wieghardt*[18] and *Chaudhuri*.[19, 20] However these complexes have a narrow range of substrates, often limited to activated alcohols and their turnover frequency remains low. Therefore, improvements with respect to catalytic activity still remain an attractive goal. Moreover, structural models for GOase are still of valuable interest. Spectroscopic data of structural models can give information about the active site of the enzyme itself.

Combinatorial chemistry has been widely used in the field of pharmaceutical chemistry.[21] More recently, its use for the discovery of new catalysts has also attracted increasing interest.[22] Peptide chemistry has been the main field of application of combinatorial synthesis. With the development of solid-phase peptide synthesis by *Merrifield*,[23] the design of the tea-bag system by *Houghten*[24] and the introduction of the split-mix method by *Furka*,[25] the automatic combinatorial synthesis of large varieties of peptides has become possible.

Towards the synthesis of structural models for natural proteins, peptides are obvious attractive candidates. This work deals with the combinatorial synthesis of a library of resin-supported peptide–copper complexes. These compounds were synthesised by the split-mix strategy and designed to mimic the active site of the enzyme GOase. The peptide–copper complexes were finally screened for activity in the catalytic aerobic oxidation of primary alcohols.

3 Background

3.1 Combinatorial chemistry and solid-phase peptide synthesis

The basic principle of combinatorial chemistry is to prepare a large number of different compounds at the same time, instead of synthesising compounds in a conventional one-at-a-time manner.[26] Various compounds are generated simultaneously under identical reaction conditions in a systematic manner, so that ideally the products of all possible combinations of a given set of starting materials (termed building blocks) will be obtained at once. The collection of these finally synthesised compounds is referred to as a combinatorial library. The library is then screened for the property in question and the active compounds are identified. In a multistep combinatorial synthesis, the total number of all synthesised compounds within the combinatorial library is determined by $N = b^x$ where b is an equal number of building blocks used in each reaction (1, 2, …, x) and x is the number of reaction steps in which a new building block is introduced. If the number of building blocks for each reaction step varies (e.g. a, b and c in a three-step synthesis), then $N = a \cdot b \cdot c$. Thus a large compound library will be obtained rapidly from only a few building blocks.

The principles used in combinatorial chemistry have their origin in the field of peptide chemistry. Solid-phase peptide synthesis was first presented by *Merrifield* in 1963.[23] Moreover, the development of the tea-bag system by *Houghten* in 1985[24] opened new possibilities for the parallel synthesis of peptides. Combinatorial synthesis of peptides was made possible by the split-mix method developed by *Furka* in 1988.[25] Since the early 1990's, combinatorial chemistry has attracted the attention of companies as a means of reducing the time and high costs associated with the traditional and serial synthesis of compounds. At first combinatorial chemistry has been mainly used in the field of drug discovery,[21, 26] but more recently its principles have been applied increasingly in the search for new materials and catalysts.[22, 27, 28]

In principle, combinatorial synthesis can be performed both in solution and on solid phase.[26] However, due to easy work-ups by simple filtrations, the possibility to increase the yields of the reaction by employing excess of reagents and the possibility of automation, the majority of compound libraries have been synthesised on solid supports such as resin beads. In the following, only the case of solid-phase combinatorial libraries will be considered.

3.1.1 Split-mix synthesis

The split-mix synthesis (also termed split-pool synthesis or divide, couple and recombined synthesis)[29-31] was first introduced by *Furka* and co-workers in 1988.[25] During the synthesis of a library by the split-mix method, the number of reaction vessels used is always inferior to the number of compounds synthesised (**Figure 3-1**).[26] In the first step, the resin is split equally in separate reaction vessels and each portion is reacted with a single building block (A_1, A_2 or A_3). After the first step has been carried out, the resin-bound compounds from all reaction vessels are pooled together in one vessel where the common steps such as resin washing and deprotection are performed. For the second step, the resin is again split in separate reaction vessels and each portion is reacted with a second building block (B_1, B_2 or B_3). The number of reaction vessels corresponds to the number of building blocks used in the step. In this way, the second solid-phase reaction provides compounds that incorporate all of the possible combinations of the two sets of building blocks. These split and mix operation are repeated until completion of the desired library. In the case of 3 building blocks used for each coupling step, a total of 27 different compounds are formed using only 9 individual reactions (**Figure 3-1**). Through this process, each resin bead in a library ends up with (ideally) just one single compound bound to it. The name of "one-bead-one-compound" for this type of libraries was introduced by *Lebl*.[30, 31]

The split-mix protocol is normally carried out on resin beads. Due to the statistical distribution of the solid support at each splitting step, the synthesis will lead to over- and under-representation within the library. In order to ensure that 95 % of all possible compound members of the library are included with a probability greater than 99 %, the split-mix synthesis should be carried out with an approximately threefold amount of resin beads.[32]
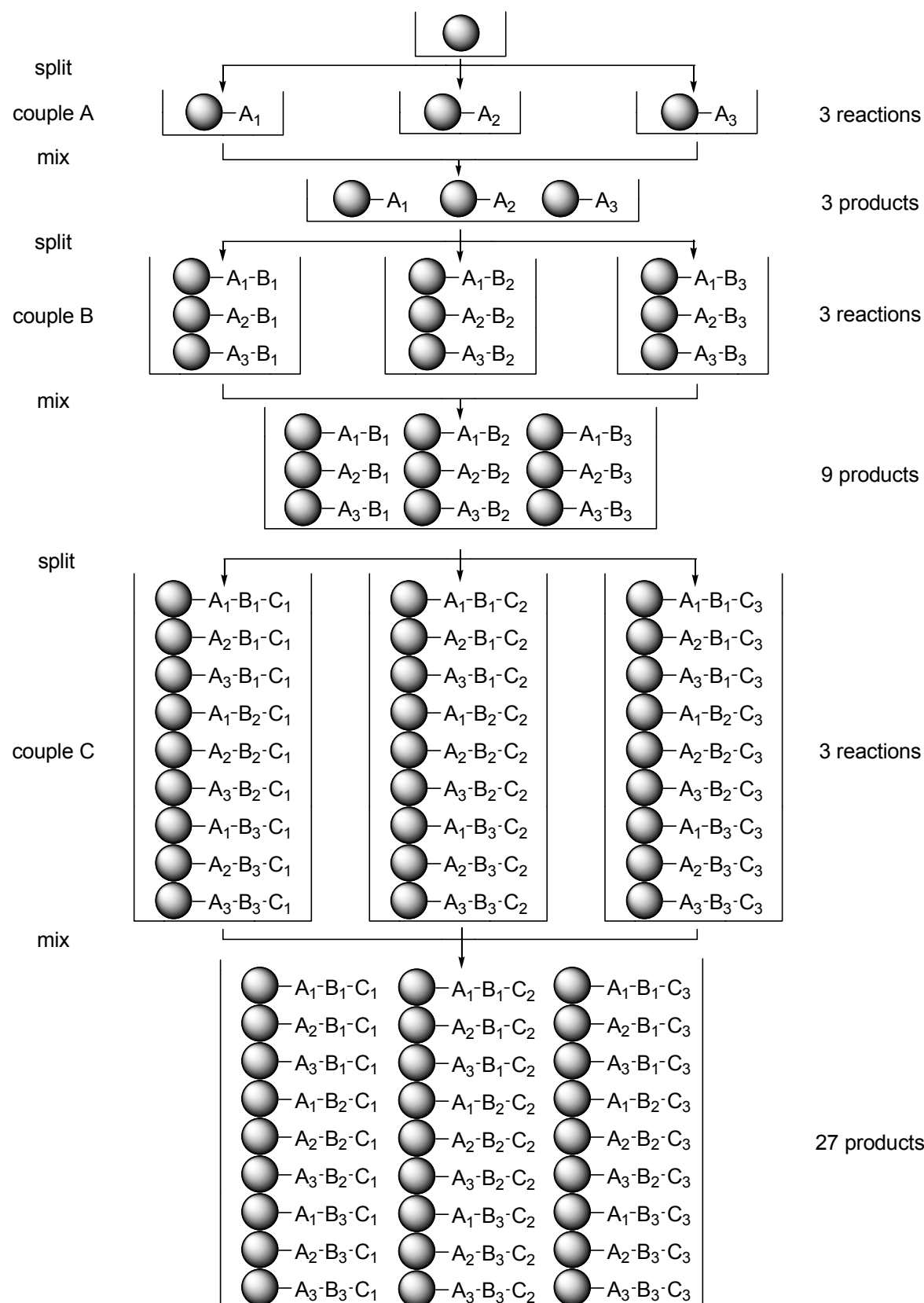
Figure 3-1: Split-mix synthesis to prepare combinatorial libraries. (Spheres represent resin beads and A, B, C represent the set of building blocks.)[26]

3.1.2   Identification of the active compounds

One of the problems arising from the split-mix synthesis of libraries is the identification or analysis of the hits. Numerous analytical systems have been developed for the quality control of combinatorial libraries comprising resin-bound compound mixture.[33] While automatic microsequencing procedures (*Edman* degradation)[34] allow the structural elucidation of peptides and oligonucleotides, mass spectroscopic methods are the method of choice for the analysis of low-molecular weight organic compounds. The sensitivity of modern mass spectrometry allows the structural determination of compounds in the high femtomolar range.[35] Especially, electrospray mass spectrometry (ES/MS) or matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) techniques are used for compound analysis from small resin samples or single beads. Recent progress has also been made in the field of IR[36] and NMR[37] spectroscopy for the analysis of small quantities of compounds.

In addition to direct methods for the structural analysis of compound libraries, there are also indirect methods, which are particularly advantageous when working with larger compound libraries made by the split-mix synthesis method. The deconvolution strategy enables the identification of active compounds of a library by synthesis and screening of several sub-libraries.[26]

An alternative strategy, termed "encoding" or "tagging", is the labelling of the compounds during the split-mix synthesis.[21, 38] The labelling can be chemical or non-chemical. In the chemical encoding strategy,[39] resin linkers containing two orthogonally protected functional groups are used to allow the concurrent synthesis of both the compound of interest and the encoding compound (so-called tags) on the bead, which upon cleavage are sequenced or otherwise decoded to determine the structure of the compound of interest (**Figure 3-2**).
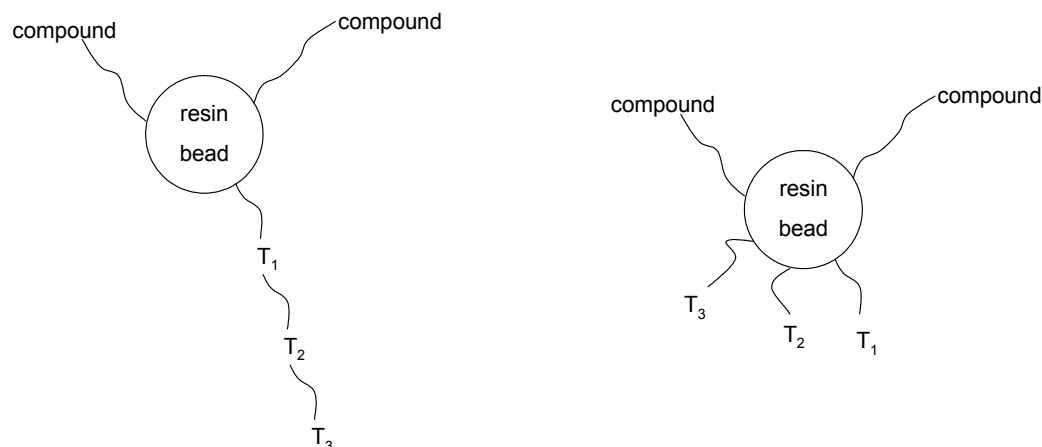
Figure 3-2: **Encoded combinatorial library by sequenceable chemical tags (left) or by non-sequenceable chemical tags (right). T_n represents the tags.**

The use of "tea-bags", associated with the radiofrequency encoding system, allows to obtain pure compounds, even when synthesised by the split-mix method. In the so-called tea-bag method, originated in 1984 by *Houghten* and co-workers,[24] the split-mix protocol occurs in 15 × 22 mm polypropylene mesh packets with μm-sized pores known as tea-bags, filled with resin beads for solid-phase synthesis. This method offers the advantage that a greater quantity of each compound of the library is available at once (up to 500 μmol), which is sufficient for a complete biological and structural characterisation. Based on the concept of the tea bags, the Kan™ reactors developed by *IRORI* are rigid containers with mesh side walls (**Figure 3-3**). These micro-reactors are available in various sizes (MiniKans, MicroKans, and MacroKans) and can be filled with resins, manually or automatically. Up to 30 mg of resin can be filled in a MicroKan and up to 300 mg in a MacroKan, leaving enough space available for the resin to swell and still remain loose within the Kan. The Kans can be closed and opened, thus allowing the removal of samples during the synthesis. The synthesis takes place by allowing the reagents to flow through the outer mesh walls of the Kan. Syntheses are performed using normal laboratory glassware and apparatus for heating, cooling, mixing, etc.
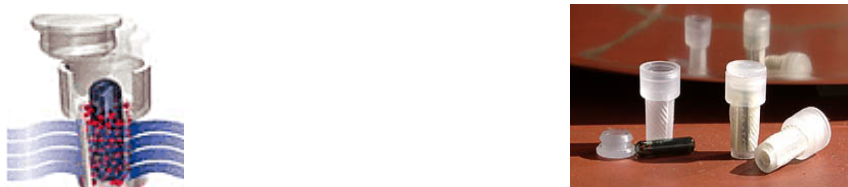
Figure 3-3: *IRORI* MacroKan containing up to 30 mg resin and a Rf tag.

More recently, *Nicolaou* and *Xiao*,[40] as well as *Moran*,[41] developed a radiofrequency encoding system. This system consists of a glass-encased semiconductor memory microchip (Rf tag) capable of receiving, storing and emitting radiofrequency signals. The use of these Rf tags together with the *IRORI* MicroKans (designed especially to accommodate a Rf tag) is of great value in split-mix protocols. Indeed, a specific radiofrequency signal is recorded on the memory microchip, and the capsules are radio-scanned between the steps of the solid-phase synthesis. Ultimately, the device can be scanned to identify the compound bound to the resin beads.

3.1.3   Solid-phase peptide synthesis

3.1.3.1 General procedure

Solid-phase peptide synthesis (SPPS) was first introduced by *Merrifield* and has become the method of choice for the synthesis of peptides and small proteins.[23]

The general approach to SPPS is outlined in **Figure 3-4**.[42] Typically, an *N*-protected α-amino acid is attached to an insoluble (solid) support via a linker. The *N*-protecting group (PG^1) is then removed and the next *N*-protected α-amino acid is coupled to the first amino acid as either a pre-activated species (symmetrical anhydride, active ester) or in situ in the presence of an activator. The cleavage/coupling cycle is repeated until the desired sequence of amino acids is obtained. The peptide is then cleaved from the linker and obtained as a free acid or amide, depending on the chemical nature of the linker. Ideally, the cleavage reagent also removes the amino acid side chain protecting goups (PG^2), which are stable under the conditions of the peptide coupling. These steps may be carried out as either a batch process, where the support is filtered between each step, or as a continuous flow process, where the support is always solvated during reagent exchange.
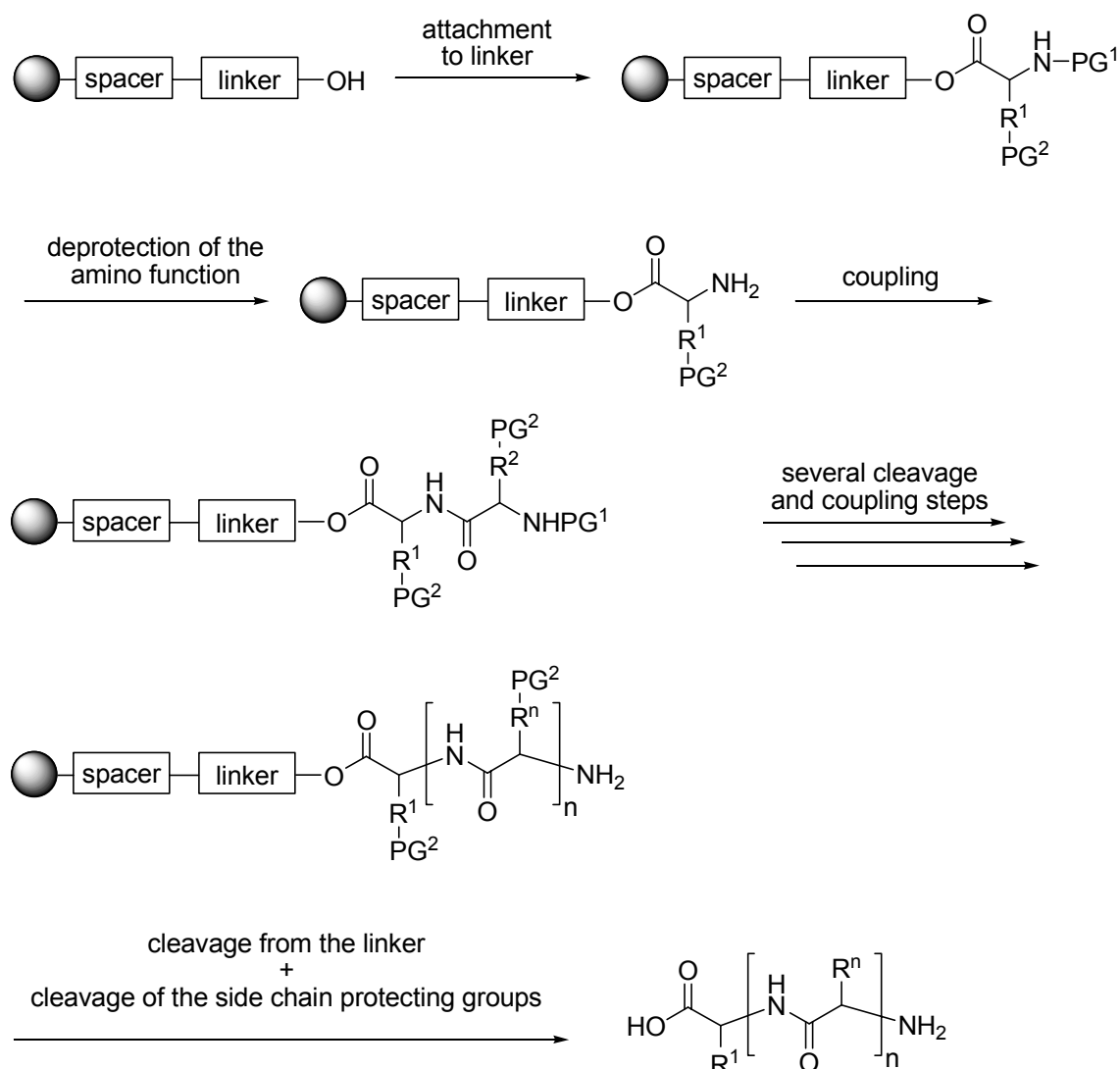
Figure 3-4: Generalised approach to solid-phase peptide synthesis. (PG^1 and PG^2 are two orthogonal protecting groups).[42]

3.1.3.2 Insoluble support

The solid support that has been most widely used in SPPS is polystyrene, cross-linked with divinylbenzene.[43, 44] The degree of cross-linking influences the ability of the resin to swell in organic solvents such as dichloromethane or DMF. The optimum resin for swelling and stability is 1 % cross-linked. Resins with a cross-linking of 0.5 % were found to be too fragile, while those with a cross-linking of 2 % or more do not swell sufficiently in dichloromethane or DMF. Based on the concept that the insoluble support and peptide chain should be of comparable polarities, other polymer supports were also used, e.g. polyacrylamide, polydimethylacrylamide, polyacrylylpyrrolidine. Moreover, controlled pore glass,

cellulose and polypropylene membranes have been demonstrated to be adequate supports.

3.1.3.3 Anchors, spacers and linkers

To allow the attachment of the first amino acid, the solid support has to be functionalised. For example, the *Merrifield* resin[42] is a chloromethylated styrene-divinylbenzene copolymer (**Figure 3-5**).
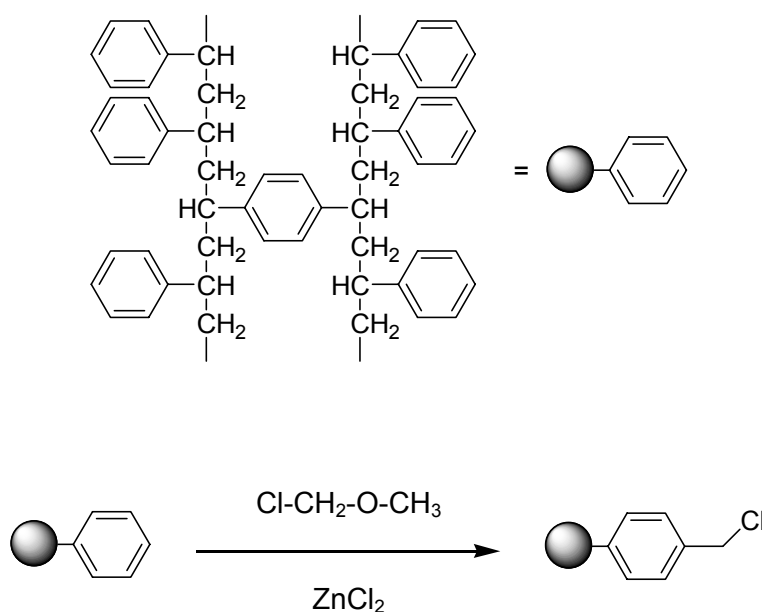
Figure 3-5: Synthesis of *Merrifield* resin by chloromethylation of a styrene–divinylbenzene copolymer.[42]

Nowadays, resins with numerous kinds of functionalities are available.[26] To the resin a linker can be bound, and if needed, also a spacer. The "spacer" is located between the resin carrier and the linker and, when present, should reduce factors such as steric hindrance of reactions brought about by the bulk of the resin or to modify features such as the hydrophilicity/hydrophobicity of the local environment. A common spacer is polyethylene glycol (PEG). The "linker" is a bifunctional molecule which, on the one hand can be bound irreversibly to the resin and, on the other hand, offers a reversible binding site for the peptide. An "anchor" is defined as a resin-immobilised functional group which forms a cleavable connection to the first building block used in the synthesis. Therefore a linker becomes an anchor after its immobilisation on the resin.

Depending on the chemical structure of the anchor and the chemistry of its attachment to the resin, the product can be cleaved at the end of the synthesis either with acid, base or a nucleophilic cleavage reagent, hydrogenolysis, enzymatic, palladium-catalysed or photochemical, oxidative and reductive cleavage methods.[26, 43]

When planning a synthesis, the type of linker or anchor has to be chosen carefully so that the peptide will stay bound to the resin until the end of the synthesis. The conditions of cleavage of the peptide from the resin have to be orthogonal to the temporary protecting groups used during the peptide synthesis.

3.1.3.4 Temporary *N*-protecting groups and side chain protecting groups

The most commonly used strategies in SPPS are the Boc/Cbz and Fmoc/*t*-Bu strategies[43] (**Figure 3-6**). In the Boc/Cbz strategy, the amino acids are *N*-protected with *tert*-butyloxycarbonyl (Boc) and the side chains are protected with benzyloxycarbonyl (Cbz), while in the Fmoc/*t*-Bu strategy, the amino acids are *N*-protected with 9-fluorenylmethoxycarbonyl (Fmoc) and the side chains are protected with *tert*-butyloxycarbonyl (*t*-Bu). The Boc protecting group is orthogonal to the Cbz protecting group since Boc is cleaved with TFA and Cbz requires stronger acidic conditions to be cleaved (HF). The Fmoc protecting group is also orthogonal to *t*-Bu since Fmoc is cleaved with secondary amines, such as piperidine, while *t*-Bu is removed under acidic conditions.

N-α-Fmoc-amino acid[43]   *N*-α-Boc-amino acid
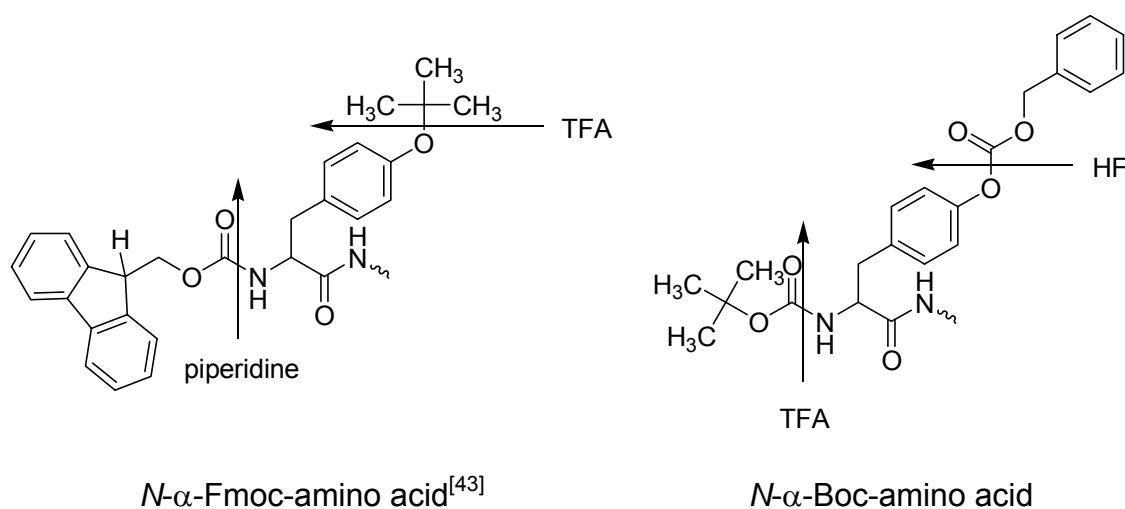
Figure 3-6: Protecting group strategies in SPPS.

In order to choose between these strategies, it is essential to use an anchor that remains stable under the conditions used for the removal of the amine protecting group. Usually, an anchor cleavable under the same conditions as the side chain protecting groups will be used. For example, a peptide is cleaved from *Merrifield* resin in strong acidic conditions (HF).[45] This resin was first used for the synthesis of peptides using *N*-Boc-protected amino acids since the anchor is stable to TFA.
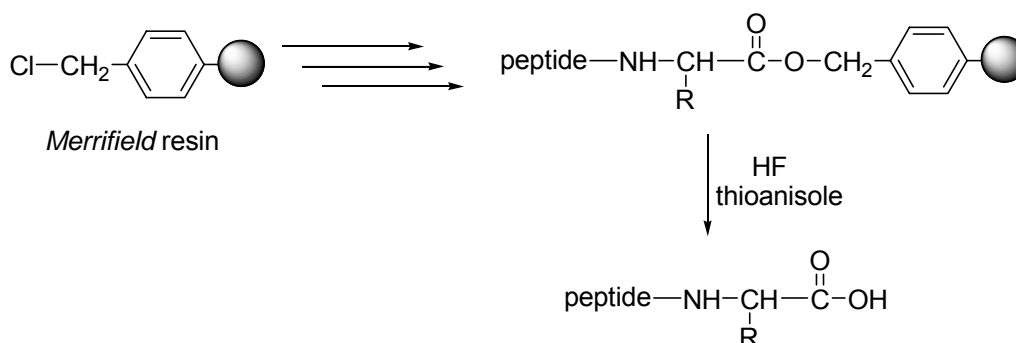
Figure 3-7: Peptide synthesis on *Merrifield* resin and cleavage with HF.[45]

The *Wang* linker is cleaved under milder conditions than *Merrifield* resin since it is cleaved with TFA.[46] This makes this linker compatible with the Fmoc/*t*-Bu strategy.
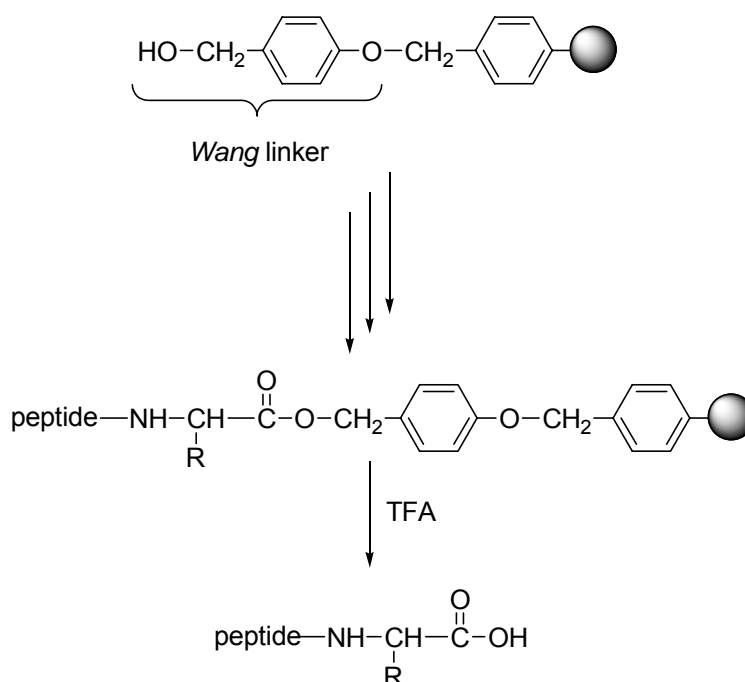
Figure 3-8: Peptide synthesis on *Wang* resin and cleavage with TFA.[46]

3.1.3.5 Coupling agents and formation of the peptide bond

For peptide bond formation, the carboxylic acid group of the *N*-protected amino acid to be coupled has to be converted into an activated species. However, over-activation should be avoided because of the risk of racemisation of the amino acid. For example, the use of acid chlorides as activated species often leads to racemisation. One of the simplest methods of peptide bond formation is the treatment of the free amino group with symmetrical anhydrides. The use of dicyclohexylcarbodiimide (DCC) for the formation of symmetrical anhydrides has been a long recognised and well-established coupling procedure in SPPS.[47] The anhydride can be prepared and latter coupled to the free amine peptide chain. Another alternative is the direct addition of the carbodiimide (DCC or DIC) and *N*-protected amino acid to the resin. However, this procedure can lead to racemisation of the amino acid, which can be suppressed by the addition of auxiliary nucleophiles such as 1-hydroxybenzotriazole (HOBt).[48] Activated esters formed from *N*-hydroxysuccinimide[49] or HOBt have been used. More recently, other activating agents have been introduced, the most famous being BOP,[50] PyBOP,[51] HBTU,[52] TSTU,[53] TBTU[54] or CDI.[55] These coupling agents are added with the *N*-protected amino acid to the resin and the activated ester is formed in situ.

DCC[47]   CDI[55]   DMAP[56]

HOBt[48]   BOP[50]   PyBOP[51]

TSTU[53]   HBTU[52]   TBTU[54]
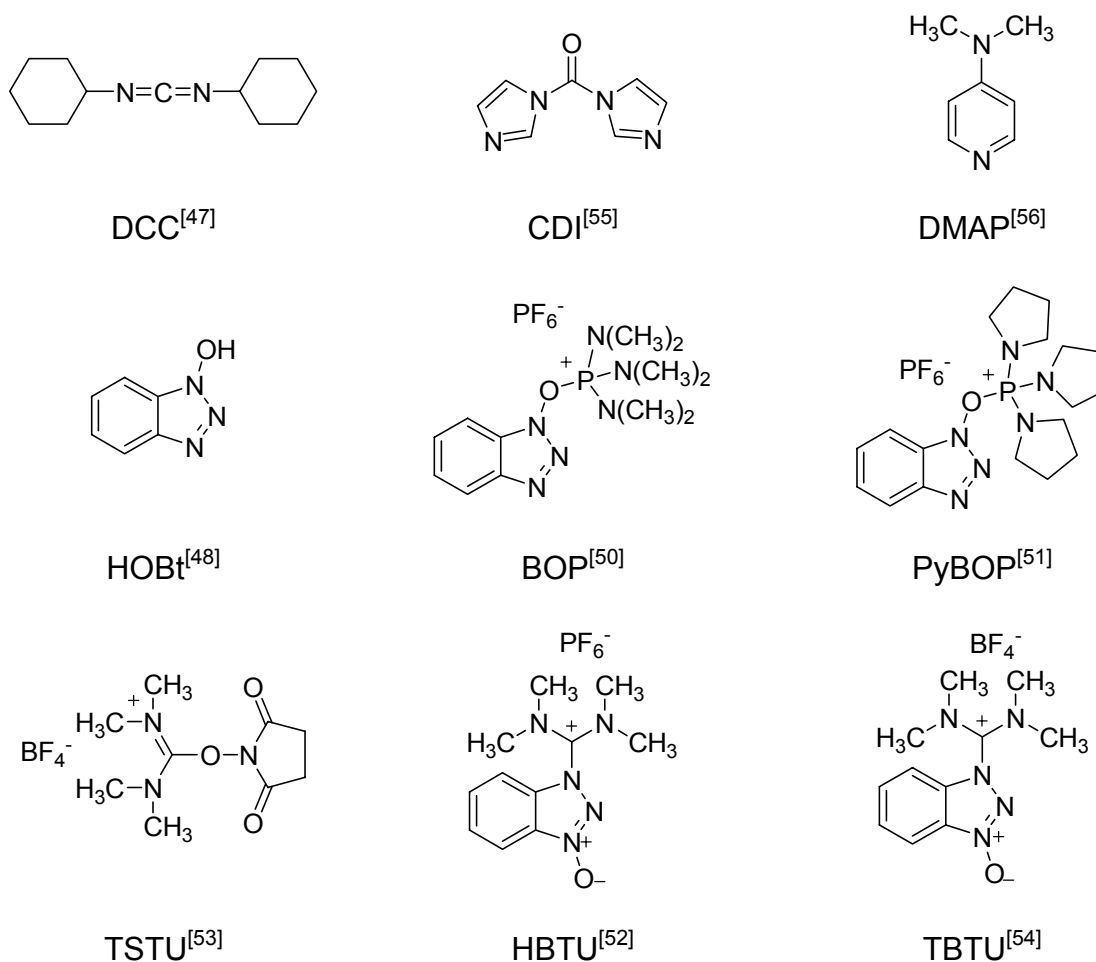
Figure 3-9: Examples of coupling agents used in solid-phase peptide synthesis. (The complete names are written in the abbreviation part.)

3.1.3.6 Monitoring

Despite the use of highly efficient coupling techniques, acylation reactions cannot be assumed to proceed with 100 % conversion. So the completion of couplings must be monitored. Monitoring techniques may be qualitative or quantitative. There are several well established qualitative monitoring methods, such as ninhydrin (Kaiser Test),[57] picric acid[58] or 2,4,6-trinitrobenzenesulfonic acid (TNBS),[59] based on reaction with free amino groups to produce a chromophoric compound.

The NF31 test, described by *De Clerq* and co-workers,[60] makes use of the *para*-nitrophenyl ester **1** (NF31) synthesised from disperse red. This colour test is performed by suspending the resin beads in a solution of **1** in acetonitrile. After heating for 10 minutes and washing, the beads containing free amino functions appear as red spheres while completely coupled beads (containing no free amino

groups) remain colourless. This visual detection is a sensitive method and is especially valuable for the detection of sterically hindered amines.
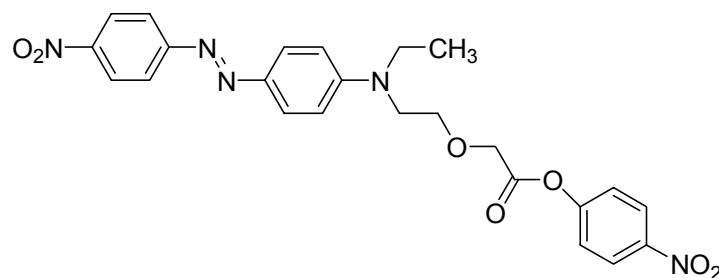
NF31, **1**[60]

Figure 3-10: Resin beads after treatment with NF31 (left: negative test; right: positive test).

SPPS using Fmoc as the *N*-protecting group offers a unique opportunity to monitor *N*-amino group deprotection. Indeed, a dibenzofulvene–piperidine adduct is formed during deprotection by piperidine.[43, 61] This chromophoric adduct has a UV absorption maximum at 301 nm. This absorption property permits reasonably accurate determination of deprotection efficiency.

UV absorption at λ = 301nm
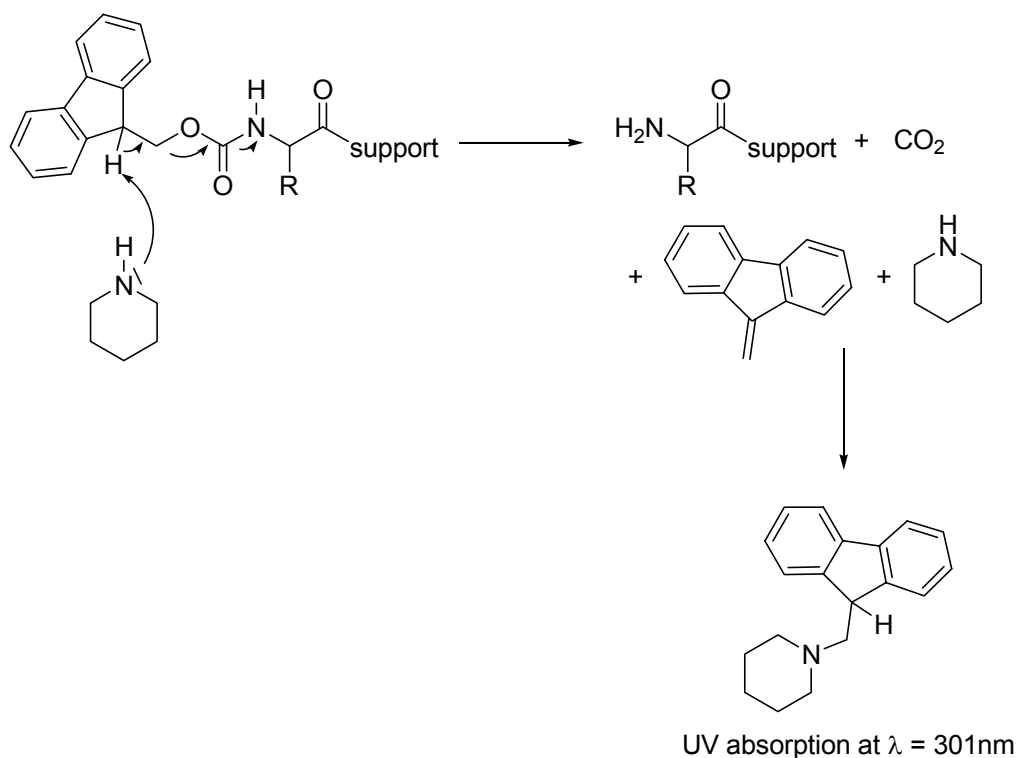
Figure 3-11: Deprotection of Fmoc amino acids by piperidine.[43, 61]

3.2   Galactose oxidase

Galactose oxidase (Goase or GO, EC 1.1.3.9) is a fungal enzyme that can be found in *Dactylium dendroides*, *Gibberella fujikuroi* or *Fusarium graminearum*.[15, 62-64] It belongs, together with glyoxal oxidase, to the family of radical-copper oxidases and it catalyses the aerobic oxidation of *D*-galactose and a wide range of primary alcohols (ranging from small molecules to polysaccharides) to the corresponding aldehydes, coupled with the reduction of dioxygen to hydrogen peroxide (**Figure 3-12**). Taking into account the low specificity of GOase for organic substrates, its biological function is probably to serve as a generator of hydrogen peroxide rather than as a source of aldehydes.[63] The specificity for oxidants is also broad: in the absence of dioxygen, several one-electron redox agents can act as electron acceptors.[63]
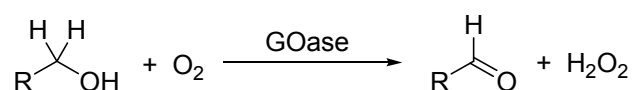
Figure 3-12: Oxidation of primary alcohols by galactose oxidase.

GOase consists of a single polypeptide with a molecular mass of 68.5 kDa. A breakthrough in the study of the enzyme came in 1991 when its crystal structure at 1.7 Å was determined by *Knowles* and co-workers.[65] The crystal structure reveals the presence of three distinct domains in the folded polypeptide: the *N*-terminal domain (residues 1 to 155) that may have the function of targeting and positioning the substrate, the catalytic domain (residues 155 to 552) and the *C*-terminal domain (residues 553 to 639).[15] The active site is a distorted square pyramidal copper complex. The equatorial positions of the complex are occupied by two histidines (His 496 and His 581), one tyrosine (Tyr 272) and a molecule of solvent. The axial position is occupied by a tyrosine (Tyr 495) (**Figure 3-13**).[65]
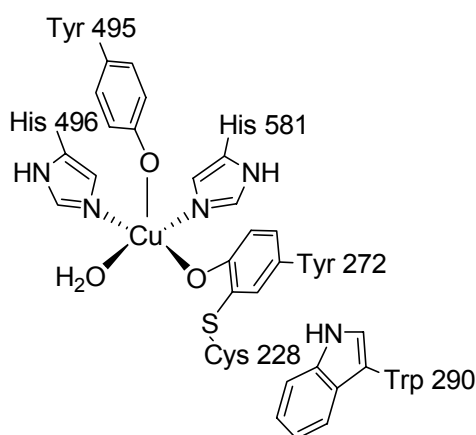
Figure 3-13:   Active site of galactose oxidase.

GOase is unusual among metalloenzymes since it catalyses a two-electron redox reaction at a mononuclear copper centre. This is made possible by the action of an organic cofactor. The equatorial tyrosine (Tyr 272) is covalently bound at the position ortho to its phenolic hydroxyl group to the sulphur atom of the adjacent Cys 228, resulting in a Tyr-Cys cross-link. This built-in cofactor serves as a one-electron redox centre since the phenol moiety can be oxidised to a phenoxyl radical during the course of the redox cycle. The interconversion between Cu(I) and Cu(II) has also been demonstrated by X-ray absorption spectroscopy.[66] So GOase can exist in three distinct, stable oxidation states. These can be assigned as highest oxidation state [Cu(II) and tyrosyl radical], intermediate oxidation state [Cu(II) and tyrosine in equilibrium with Cu(I) and tyrosyl radical] and lowest oxidation state [Cu(I) and tyrosine] (**Figure 3-14**).[64] The highest oxidation state is the catalytically active form

of the enzyme. This active oxidised form is EPR-silent, indicating that the cupric ion is antiferromagnetically coupled to the tyrosyl radical.
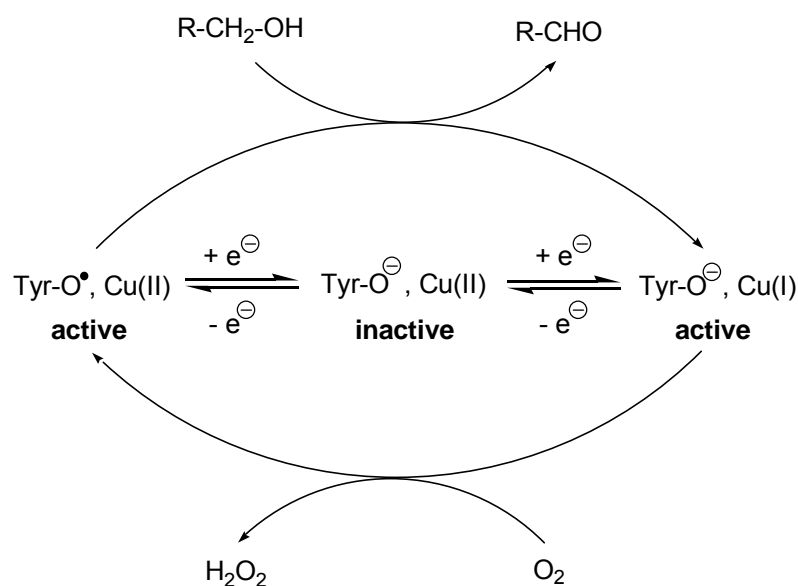
Figure 3-14: **Redox interconversion of galactose oxidase species.**

Moreover, the Tyr-Cys moiety is π-stacked to a tryptophan residue (Trp 290) (**Figure 3-13**). The interaction between Tyr-Cys and tryptophan side chains is believed to have a significant stabilising effect on the Tyr-Cys redox couple, even though there is no evidence for delocalisation of the radical over the indole moiety. The role of the tryptophan is probably to protect the cofactor from the solvent.[15] Another interesting feature of the active site is the direct backbone link between axial Tyr 495 and equatorial His 496.

3.2.1   A radical mechanism

Already before the availability of the X-ray structure of the enzyme, *Whittaker* detected the tyrosyl radical and proposed a radical mechanism for the turnover of GOase.[67] Taking advantage of the structural data from X-ray crystallography and kinetic evidence with radical-probing substrates, *Branchaud* and co-workers proposed a more detailed mechanism.[68] Then *Whittaker* refined the mechanistic scheme of *Branchaud* and proposed that Tyr 495 could act as a base to deprotonate the alcohol substrate.[69] Although there are some minor differences in the proposed mechanisms, the central feature of them all is that enzymatic catalysis was proposed

to proceed by a stepwise radical mechanism with a substrate-derived ketyl radical as a key intermediate.

Figure 3-15 shows the mechanism that has been generally accepted. First, the substrate binds to the equatorial copper position (occupied by water or acetate in the crystal structures) to give copper complex **B**. Then a proton transfer from the alcohol to the axial tyrosinate (Tyr 495) leads to compound **C**. Next, in a step known from isotope substitution experiments to be at least partially rate-limiting and probably the major rate-limiting step, a hydrogen atom is transferred from the substrate to the equatorial modified tyrosyl radical (Tyr 272), giving **D**. The resulting substrate derived ketyl radical is then oxidised through electron transfer to the copper centre yielding Cu(I) and the aldehyde product (**E**). Cu(I) and tyrosine are, finally, reoxidised by molecular oxygen through complex **F**, regenerating Cu(II) and tyrosyl radical, and giving hydrogen peroxide as a product. The hydrogen peroxide, which is a poor ligand, is replaced by a molecule of substrate and complex **B** is formed again.

Further studies from the groups of *Branchaud*,[64] *Whittaker*[70] and *Himo*[71] have confirmed the proposed mechanism and gave more insight into the enzyme's oxidation process.

Branchaud and co-workers used β-haloethanol to trap the enzyme in a catalytically inactive one-electron reduced form.[64] *Himo* and co-workers used density functional methods and molecular mechanics to study the catalytic mechanism of GOase.[71] Their calculations strongly support the experimentally proposed mechanism, with one exception. They proposed that prior to the initiating proton transfer step the radical is located at the axial tyrosine (Tyr 495) and not at the equatorial thioether-substituted Tyr 272. The transfer of the proton and the one of the radical to Tyr 272 probably happens simultaneously. In 2001, *Whittaker* reported kinetic studies on a homologous series of benzyl alcohol derivatives.[70] These results support a mechanism in which initial proton abstraction from a coordinated substrate activates the alcohol towards inner sphere electron transfer to the Cu(II) metal centre in an unfavourable redox equilibrium, forming an alkoxy radical which undergoes hydrogen atom abstraction by the Tyr-Cys phenoxyl free radical ligand to form the product aldehyde. In another report, *Whittaker* concludes that the hydrogen atom transfer must be the first step, but that the substrate oxidation is a single-barrier process without resolved kinetic intermediates.[15]
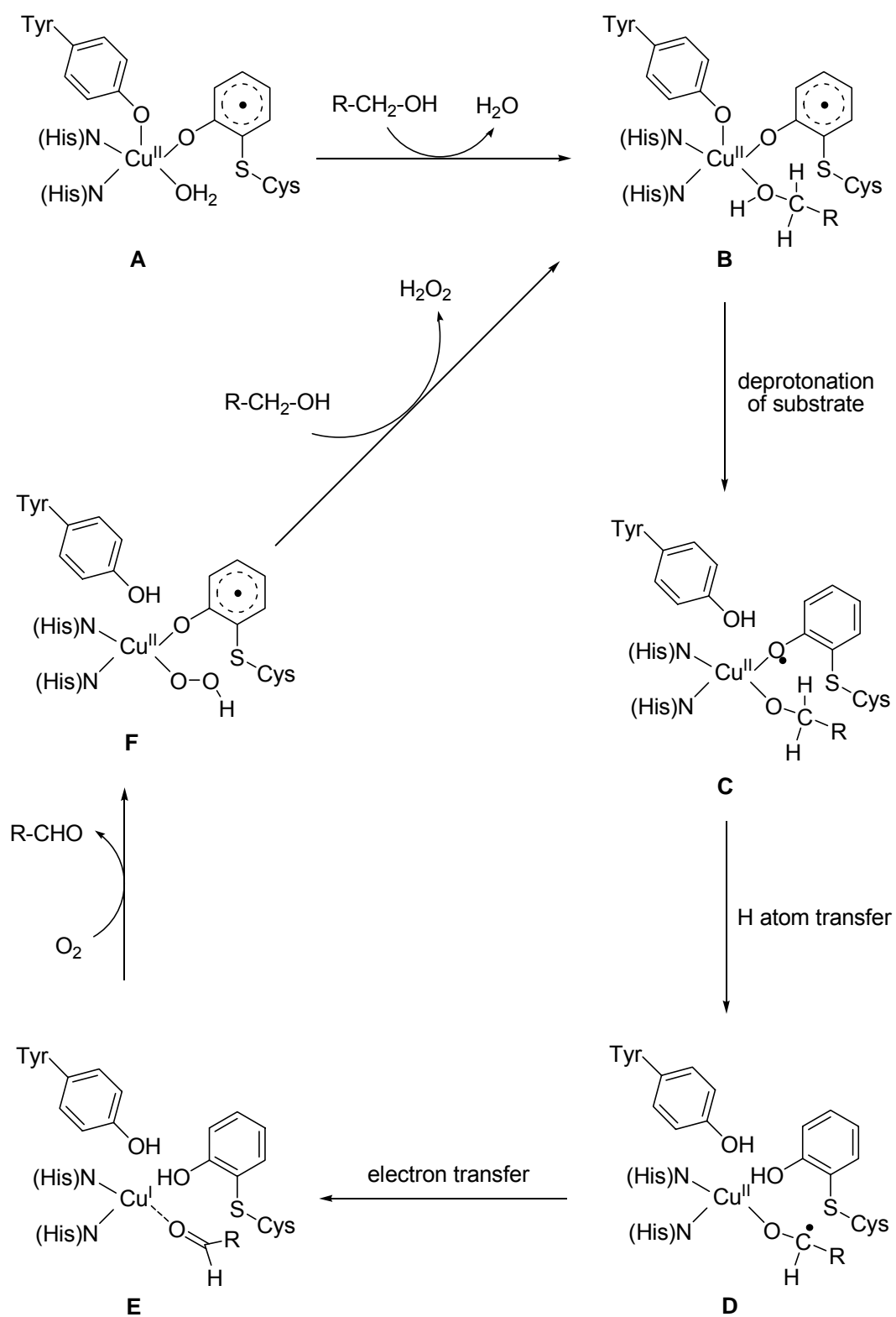
Figure 3-15: Proposed mechanism for the aerobic oxidation of primary alcohols by galactose oxidase.

3.2.2 Structural models for studying the reactivity of GOase

Model complexes of the active site of galactose oxidase have been synthesised by various groups with the aim of providing insight into the mechanism of the enzyme.

One of the interesting features of the enzyme is the existence of the thioether linkage between Tyr 272 and Cys 228. A question that has been asked is why galactose oxidase employs such modified amino acid residues instead of simple tyrosine. The redox potential of galactose oxidase is estimated to be 400–500 mV versus NHE,[67] which is significantly lower than that of free tyrosine in solution (930 mV) or tyrosine in enzymatic systems (760–1000 mV).[72] Such a negative shift of the redox potential can be attributed to both the electron-donating nature and the radical-stabilising effect by electron spin delocalisation into the methylthio group, so-called electron-sharing conjugative effect. Synthetic models from *Fukuzumi*[73-75], *Whittaker*[76, 77] and *Tolman*[78] have brought detailed insight into the electronic effects of the thioether group of the cofactor on the physicochemical properties and the reactivity of the Cu(II)–phenoxyl radical species. Furthermore, the π-π-stacking interaction of the cofactor with the tryptophan (Trp 290) side chain seems to play a crucial role in the catalytic cycle, even if its role is not yet really defined. With the goal of clarifying the role of the tryptophan, *Halcrow* has synthesised structural models of GOase containing aromatic moieties able to show π-π interaction with the phenolate moiety.[79, 80] In this section, a few examples of ligands and copper complexes synthesised to provide insight into the mechanism of GOase are given.

Fukuzumi, *Itoh* and co-workers have examined the physicochemical properties of the ligands **2** and **3**, together with cresol as a reference.[73, 75] ^{1}H NMR data of these compounds reveal the electron-donating nature of the thioether substituent. Moreover, the thioether substituent causes a negative shift of the redox potential (1106 mV for ligand **2** and 955 mV for ligand **3** compared to 1474 mV for *para*-cresol). Analysis of the radical species of compounds **2** and **3** by UV spectroscopy, cyclic voltammetry and ESR revealed behaviour of the ligands very similar to the behaviour of the oxidised form of GOase.
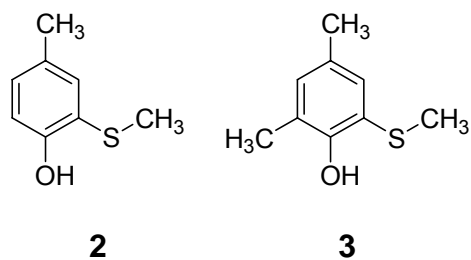
The first copper(II) complex model for the active site of the enzyme was reported by *Whittaker* and co-workers in 1993.[76] This model **4** consists of the ligand **2** and *N,N,N',N'',N''*-pentamethyldiethylenetriamine complexed with a copper(II) ion. The copper complex has a square pyramidal structure. However, the sulphur atom of the methylthio group coordinates to the copper ion from the axial position, making the coordination geometry different from that of GOase where there is no coordinative interaction between the sulphur atom and Cu(II). Treatment of **4** with refluxing acetonitrile gave an ortho–ortho coupled dimer of **2**, indicating an inner-sphere electron transfer from the phenolate to Cu(II) to generate a phenoxyl radical and Cu(I) (**Scheme 3-1**).
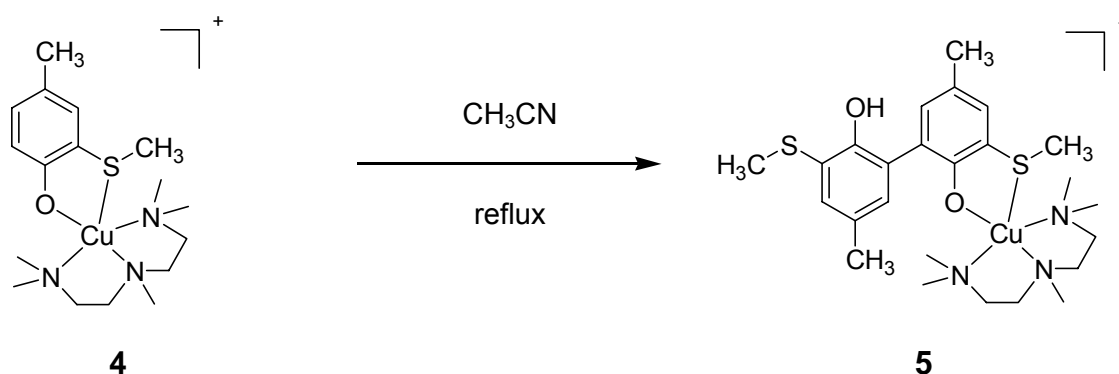
Scheme 3-1: Refluxing *Whittaker*'s copper(II) complex in acetonitrile gives a dimer.[76]

Later, *Whittaker* and co-workers have synthesised ligand **6** in order to mimic the N_2O_2 donor set of GOase.[77] A copper(II) complex of this ligand was obtained in a dimeric form containing a $Cu(II)_2O_2$ core as a linker group. The dimeric complex could be converted into the monomer by adding an external ligand such as pyridine. The copper monomer showed an ESR spectrum typical for a distorted square pyramidal structure. Studies by cyclic voltammetry of the copper monomer showed

irreversible oxidation waves at 434 mV and 781 mV versus NHE due to the one-electron oxidation of the phenolate group.

Similar observations were made by *Fukuzumi* and co-workers with ligands **7** and **8**. The dimeric complex is formed and can be converted to the monomeric copper(II) complex. The ESR spectrum of the monomeric complex is typical for a square pyramidal geometry.[73] In cyclic voltammetry, ligand **7** gave a reversible wave at 688 mV versus NHE while ligand **8** which does not contain any thioether group did not show any reversible wave.

6   **7**   **8**

$[Cu(II)_2(7^-)_2]^{2+}$ $Cu(II)(7^-)(Py)^+$
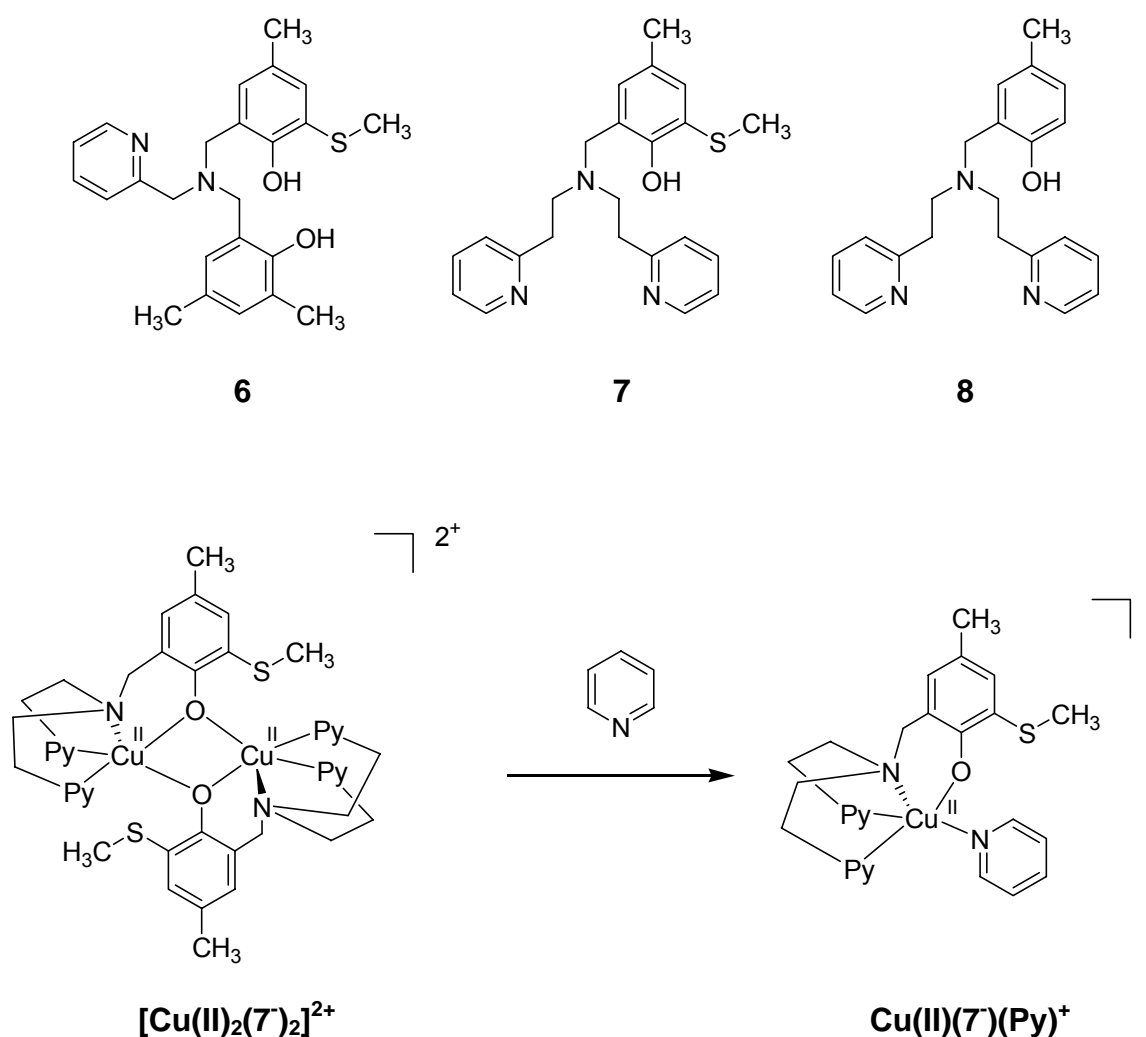
Scheme 3-2: *Fukuzumi*'s dimeric and monomeric copper complexes of ligand **7**.[75]

Tolman and co-workers reported a copper(II) complex with a 1,4,7-triazacyclononane derived ligand.[78] Complex **9** was isolated with an alcohol molecule coordinated to it. Bulk electrolysis of the benzyl alcohol adduct (398 mV versus NHE in acetonitrile)

produced benzaldehyde in 46 % yield. It is interesting to note that there is a hydrogen bonding interaction between one of the benzylic protons of the bound substrate and the phenolate oxygen of the "cofactor".
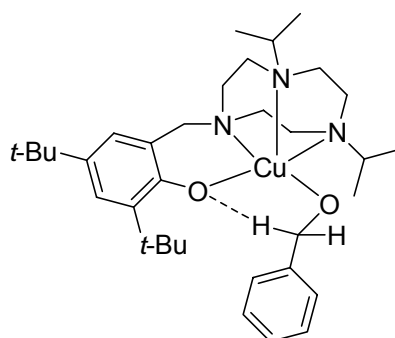
9

In order to demonstrate the role of the *ortho*-thioether substituent as well as the role of the π-π interaction present at the active site of GOase, *Halcrow* and co-workers synthesised the compounds 3,4-benzo-8,9-(3'-hydroxybenzo)-bicyclo[4,4,1]-undeca-3,8-dien-11-one **10** and 3,4-benzo-8,9-(3'-hydroxy-4-methylsulfanylbenzo)-bicyclo-[4,4,1]-undeca-3,8-dien-11-one **11**, as well as their ethylene acetals (**12** and **13**).[79] Crystallographic, UV–vis and NMR studies showed that the two ketones adopt boat/chair conformations, while the acetals exhibit chair/chair conformations with layered aryl rings. Analyses by cyclic voltammetry showed that the methylsulfanyl substituent and the π-π interaction both lower the oxidation potential of the phenol ring in these compounds. A difference of 0.29 V between the oxidation potential of compound **10** and compound **12** and a difference of 0.15 V between the oxidation potential of compound **11** and compound **13** imply that the π-π interaction contributes to the thermodynamic stability of the oxidised species. Compound **13** exhibits an oxidation potential 0.46 V lower than that of **10**. This mirrors almost exactly the thermodynamic stabilisation of the GOase Tyr-Cys phenoxyl radical, which contains an *ortho*-thioether substituent and an overlying π-π interaction.

10　　　　　**11**　　　　　**12**　　　　　**13**
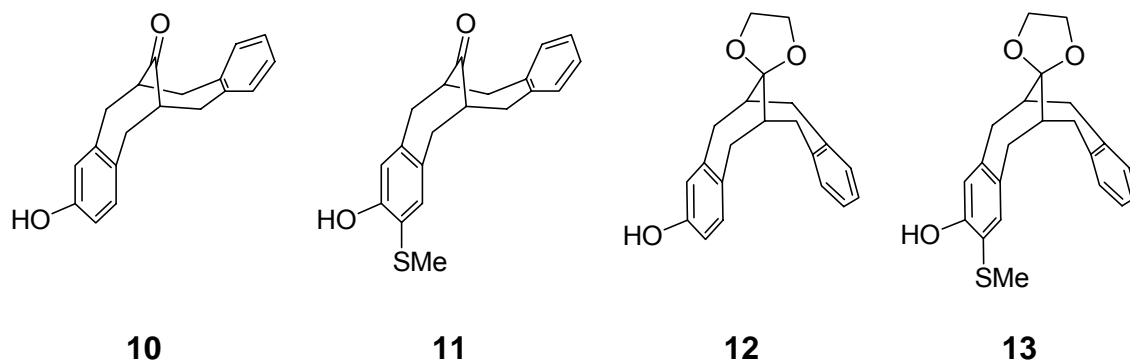
3.3 Copper catalysed oxidation of alcohols and active models for galactose oxidase

Groups from both the industrial and the academic research have been working more and more on the development of green oxidation catalysts. Their main goals are the reduction of waste and the reduction of costs of production. In the field of alcohol oxidation, we have seen the apparition of new metal catalysts that use molecular dioxygen as a stoichiometric oxidant.[9, 12] These are particularly attractive since the only byproduct is water or hydrogen peroxide. Besides palladium[7] and ruthenium,[8] copper has also been used as a metal for the aerobic oxidation of alcohols.[17]

3.3.1 Catalytic oxidation with TEMPO

One of the most frequently applied methods for the catalytic oxidation of alcohols using the stable free radical 2,2',6,6'-tetramethylpiperidine-*N*-oxyl (TEMPO, **14**) does not employ oxygen as a stoichiometric oxidant but sodium hypochlorite (NaOCl) or bleach (**Scheme 3-3**).[81] In this reaction, the corresponding oxoammonium salt **15** is the active oxidant (obtained by *in situ* oxidation of **14** by NaOCl or NaOBr). Oxidation of the alcohol affords the corresponding carbonyl compound and the reduced form of TEMPO, the hydroxylamine **16**. The latter is then re-oxidised by NaOCl or NaOBr to generate the oxoammonium salt **15**. In the generally applied procedure,[82] sodium bromide (10 mol%) is used as a cocatalyst, since the re-oxidation step is more favourable with NaOBr and the sodium bromide produced is readily re-oxidised by NaOCl (**Figure 3-16**).

TEMPO, **14**

R^1, R^2 = aryl, alkyl

75–95 %
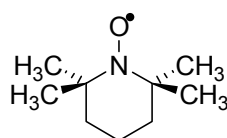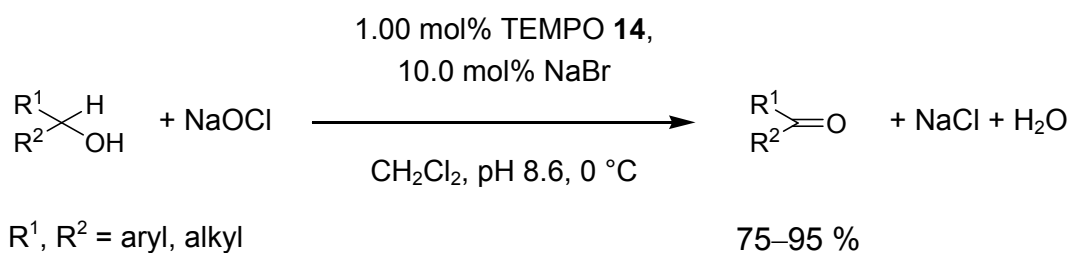
Scheme 3-3: **Catalytic oxidation of alcohols with TEMPO and sodium hypochlorite as a stoichiometric oxidant.**[82]
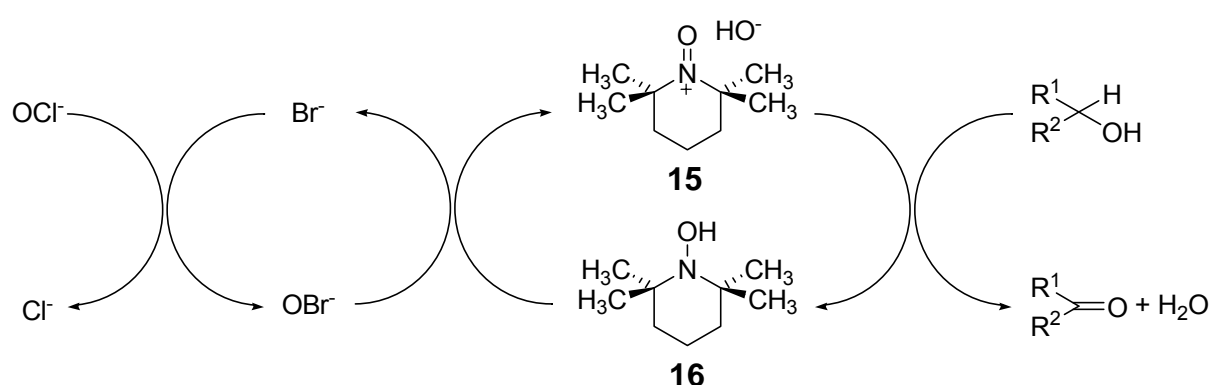
Figure 3-16: **TEMPO-catalysed bleach-oxidation of alcohols using bromide as a cocatalyst.**[83]

A shortcoming of this method is that it is not waste free since one equivalent of NaCl is produced per molecule of alcohol oxidised.

Alternatively, the use of TEMPO in combination with copper salts and oxygen as a primary oxidant was first reported by *Semmelhack* in 1984.[10] At room temperature, allylic and benzylic alcohols are oxidised by molecular oxygen using a catalytic mixture of cuprous chloride and nitroxyl radical **14** in DMF. In this experiment, 10 mol% of CuCl and 10 mol% of TEMPO **14** or its 4-hydroxy analogue **16** are used to convert benzyl alcohol to benzaldehyde in 2 hours with bubbling of dioxygen through the solution. Aliphatic alcohols do not give complete conversion under these

conditions. A first catalytic cycle was proposed by *Semmelhack* for this reaction where copper(II) oxidises the nitroxyl radical **14** to the oxoammonium **15**. The oxoammonium **15** is the species able to perform the oxidation of alcohols, giving the corresponding aldehydes, together with the hydroxyl compound **16**. The hydroxyl compound **16** and the oxoammonium **15** react stoichiometrically to give the starting material TEMPO **14**. Further, the copper(I) species is re-oxidised to copper(II) by molecular dioxygen.

$$R\text{-}CH_2\text{-}OH \xrightarrow[\text{DMF, 2 h, O}_2]{\substack{\text{10.0 mol\% TEMPO } \mathbf{14},\\ \text{10.0 mol\% CuCl}}} R\text{-}CHO$$

R = aryl, vinyl   90–100 %
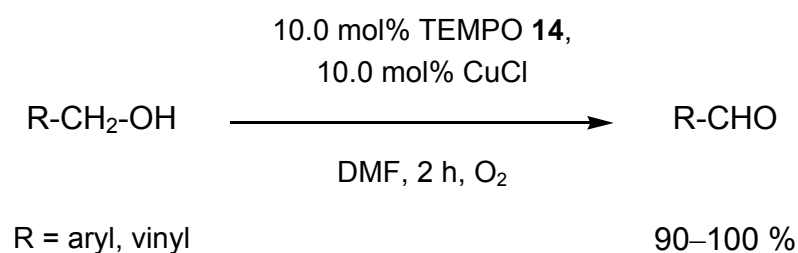
Scheme 3-4: Catalytic aerobic oxidation of alcohols with TEMPO and CuCl.[10]
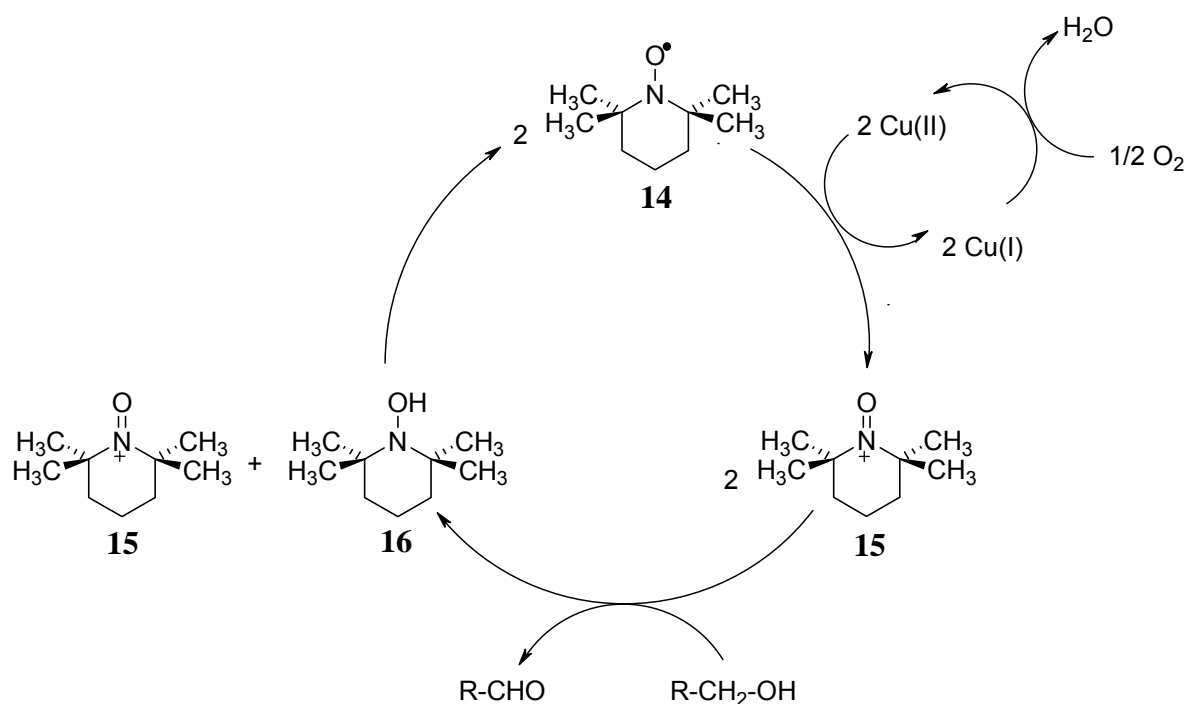
Figure 3-17: Catalytic cycle for the aerobic oxidation of primary alcohols with TEMPO according to *Semmelhack*.[10]

In 2003, *Sheldon* and co-workers reported Hammett correlation studies and primary kinetic isotope effect studies for this CuCl-TEMPO catalysed aerobic oxidation of benzylic alcohols.[84] These results are inconsistent with a mechanism where the active species is the oxoammonium cation **15**. Instead, *Sheldon* proposed a copper-mediated dehydrogenation mechanism in which the active copper(II) species is generated via a one-electron oxidation of copper(I) by TEMPO **14** (**Figure 3-18**). Alkoxy replacement, followed by coordination of a second molecule of TEMPO and intramolecular β-hydrogen abstraction afford the desired carbonyl compound, Cu(I) and TEMPOH **16** (**Figure 3-19**). Finally TEMPO **14** is regenerated by rapid air oxidation of TEMPOH **16**. This mechanism is analogous to the one formulated for the enzyme galactose oxidase.
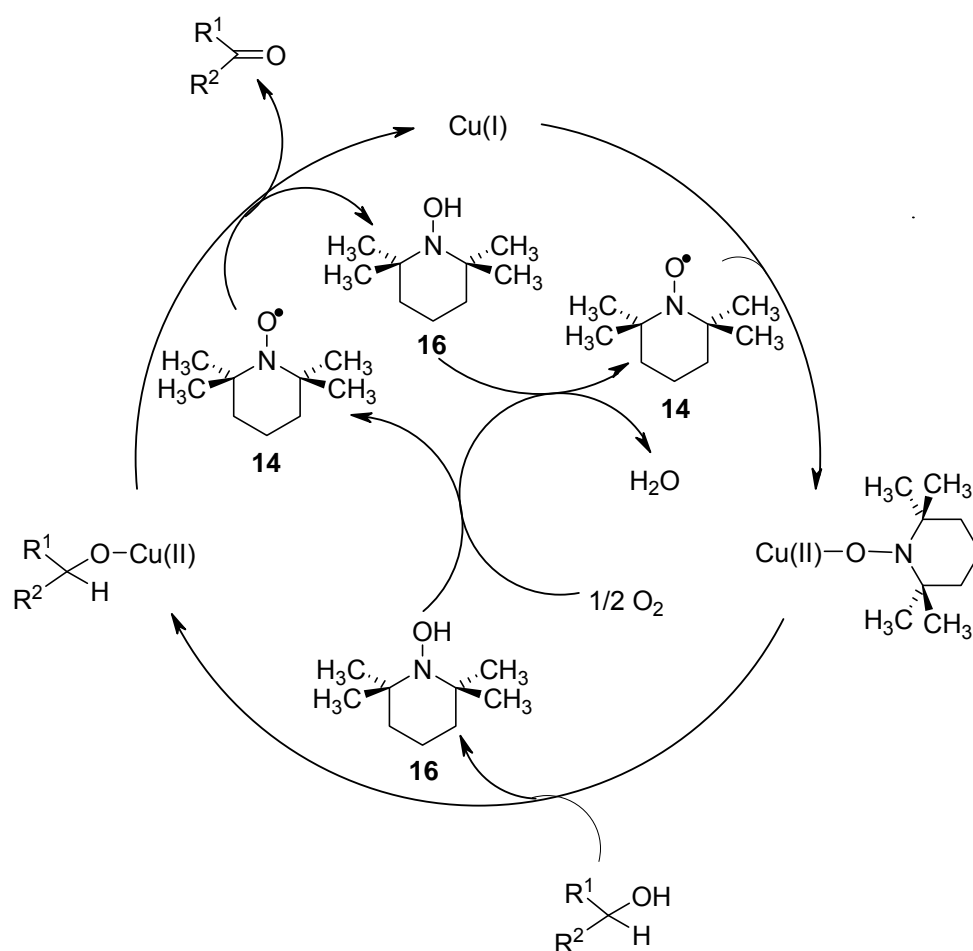
Figure 3-18: Mechanism postulated by *Sheldon* for the CuCl-TEMPO catalysed aerobic oxidation of activated alcohols.[84]
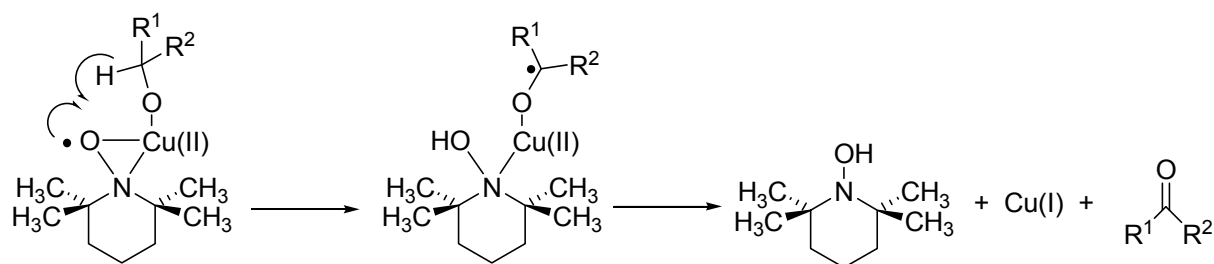
Figure 3-19: Intramolecular transfer of β-hydrogen, followed by oxidative elimination.[84]

Recently, *Sheldon* and co-workers reported a mild catalytic oxidation where primary alcohols are selectively converted to aldehydes.[9] In this novel procedure, $CuBr_2$(2,2'-bipyridine) catalyses the aerobic oxidation of primary alcohols in acetonitrile : water (2 : 1) in the presence of TEMPO and a base as a cocatalyst. Typically, the reaction is performed at room temperature with 5 mol% of $CuBr_2$, 5 mol% of 2,2'-bipyridine, 5 mol% of TEMPO and 5 mol% of potassium *tert*-butylate.

Under these conditions, various benzylic, allylic and aliphatic alcohols have been converted into their corresponding aldehydes. The oxidation of benzyl alcohol proceeded with a turnover frequency of 14 h^{-1} which is an excellent result for a room temperature oxidation reaction involving a copper catalyst. Secondary alcohols were unreactive under these conditions. In this reaction, TEMPO may act as a hydrogen acceptor during the catalytic cycle as in the CuCl-TEMPO catalysed oxidation (**Figure 3-19**). In the case of activated alcohols (benzylic and allylic), the oxidation is faster than in the case of aliphatic ones, indicating that hydrogen abstraction from the α-carbon atom by TEMPO is the rate-determining step of the reaction. The poor reactivity of secondary alcohols could be explained in terms of steric hindrance. In addition, in the case of primary alcohols, the second β-hydrogen atom is bonded to the oxygen atom of TEMPOH, stabilising the radical intermediate (**Figure 3-20**).

5.00 mol% CuBr$_2$, 5.00 mol% bipyridine ,
5.00 mol% TEMPO **14**, 5.00 mol% *t*-BuOK

R-CH$_2$-OH → R-CHO

CH$_3$CN/H$_2$O (2:1), air

R = aryl, vinyl, alkyl
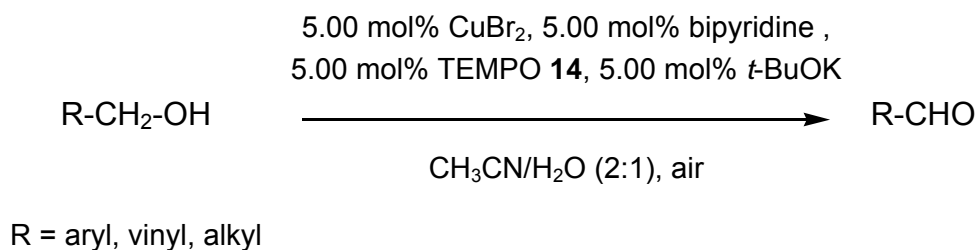
alcohol	time [h]	conversion [%]
benzyl alcohol	2.5	100
geraniol	5	100
octan-1-ol	24	61
octan-2-ol	5	no reaction

Scheme 3-5: Catalytic aerobic oxidation of alcohols with TEMPO and [CuBr$_2$(2,2'-bipyridine)] according to *Sheldon*.[9]
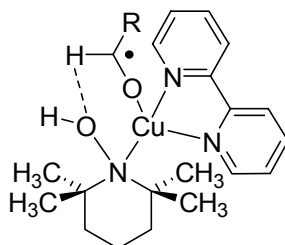
Figure 3-20: Stabilisation of the radical species by the second β-hydrogen of primary alcohols.[9]

3.3.2 Oxidation with azo compounds

The catalytic aerobic oxidation of alcohols with copper chloride, phenanthroline and an azo compound such as diethylazodicarboxylate (DEAD) or its *tert*-butyl analogue (DBAD) was reported by *Markó* and co-workers for the first time in 1996.[11] With 5 mol% of CuCl, 5 mol% of phenanthroline, 5 mol% of DBAD or its reduced form DBADH$_2$, 25 mol% of K$_2$CO$_3$ and dioxygen or air in fluorobenzene at 80 °C, primary allylic and benzylic alcohols, as well as secondary aliphatic, allylic and benzylic alcohols can be oxidised to the corresponding aldehyde or ketone in 80 % to 100 %

yield in 1 to 2 hours.[85] However primary aliphatic alcohols are unreactive under these conditions. Later, *Markò* improved the reaction conditions which led to a catalytic system that quantitatively oxidises a large variety of primary alcohols to the corresponding aldehydes.[12] The addition of a heterocyclic amine is necessary to promote the oxidation of primary aliphatic alcohols to aldehydes, with *N*-methylimidazole giving the best results (**Scheme 3-6**).

$$\text{R-CH}_2\text{-OH} \xrightarrow[\text{C}_6\text{H}_5\text{F, O}_2,\ 70\text{–}80\ °\text{C, 100 min}]{\substack{\text{5.00 mol\% CuCl/phen, 5.00 mol\% DBAD}\\\text{5.00 mol\% }t\text{-BuOK, 7.00 mol\% NMI}}} \text{R-CHO}$$

R	yield [%]
C_9H_{19}	95
	93
	95
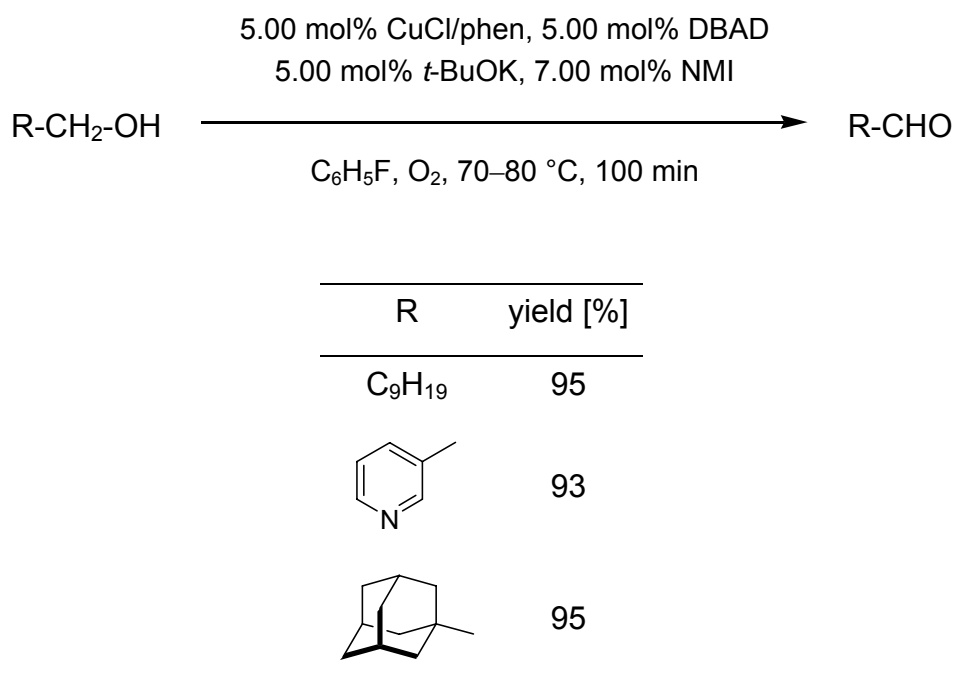
Scheme 3-6: Copper-catalysed aerobic oxidation of primary alcohols according to *Markó*.[12]
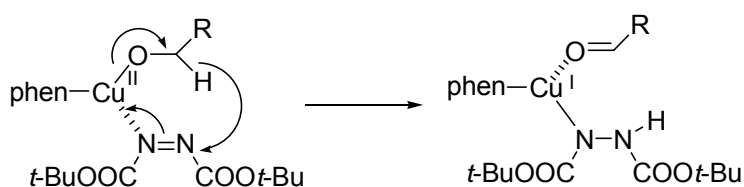
Figure 3-21: Proposed intermediate for the copper-catalysed aerobic oxidation of alcohols according to *Markó*.[12]

So far this protocol is the only copper-containing system that catalyses the aerobic oxidation of all classes of alcohols, including primary aliphatic alcohols. No over-oxidation to carboxylic acids has been observed. However, this system still

suffers from two major drawbacks: the oxidation has to be carried out at temperatures above 70 °C and the product obtained needs to be purified by column chromatography. Easy isolation of the product and recycling of the catalyst have to be taken into consideration for the synthesis of future generations of catalysts.

3.3.3 Structural and active models of galactose oxidase

Stack and co-workers described the salen type–copper(II) species with a binaphthyl backbone **17**-**22**.[16, 86] Copper complexes **17** and **21** show catalytic activity, while copper complexes **18**, **19**, **20** and **22** do not show any catalytic activity. This implies that a 3,5-disubstitution on the salicylate ring is necessary for the catalytic activity. The copper complex **17** catalyses the oxidation of neat benzylic and allylic alcohols under 1 atmosphere of dioxygen at room temperature (**Scheme 3-7**). Due to the phenol moieties substituted with a thioether, this ligand is a structural model of GOase. With as little as 0.01 mol% of the salen type–copper(II) catalyst and 2 mol% of a base, neat benzyl alcohol was converted to benzaldehyde in 20 hours, resulting in a turnover of 1300. Moreover, the copper-complex with a thioether linkage **17** is more active than the copper-complex with two *tert*-butyl substituents **21**.

The rate determining step of this system was suggested to involve abstraction of the benzylic hydrogen, resulting in a ketyl radical. Then an inner sphere one-electron transfer from the ketyl radical to the copper(II) results in copper(I), phenol and the carbonyl compound (**Figure 3-23**). The copper complex is then reoxidised by dioxygen which is reduced to hydrogen peroxide. This mechanism is analogous to the one observed in the enzyme galactose oxidase.

R^1	R^2	complex
SPh	t-Bu	17
Si-Pr	t-Bu	18
H	t-Bu	19
SPh	H	20
t-Bu	t-Bu	21
H	H	22
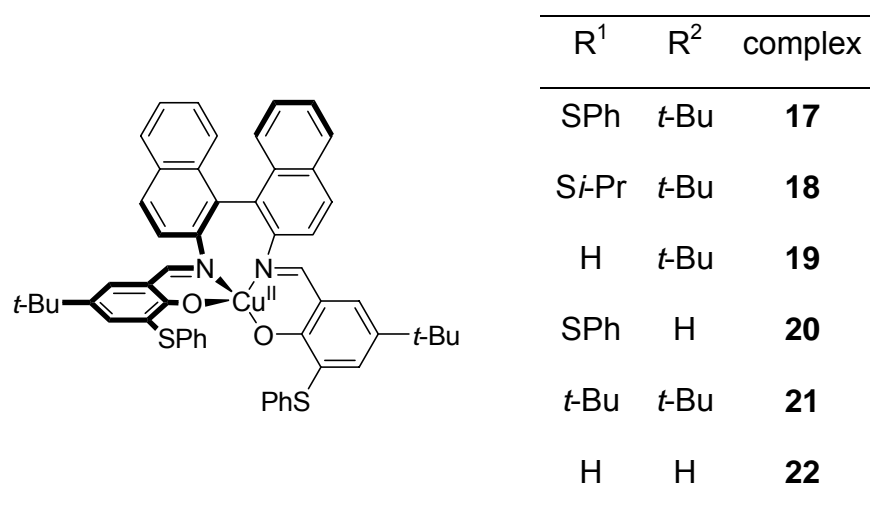
Figure 3-22: Salen-type copper(II) complexes by Stack.[86]
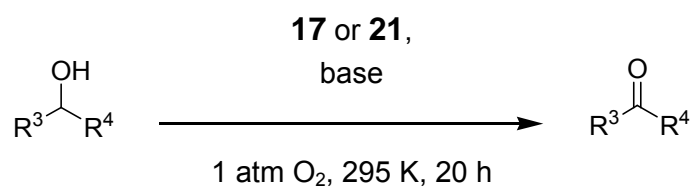
R^3	R^4	complex		base	TON
			[mol %]	[mol %]	
Ph	H	17	0.01	2.00	1300
Ph	H	21	0.01	2.00	200
Ph	CH$_3$	17	0.05	0.30	60
Ph	CH$_3$	21	0.05	0.30	30

Scheme 3-7: Copper-catalysed aerobic oxidation of primary alcohols with Stack's copper(II) complexes 17 and 21.[16]
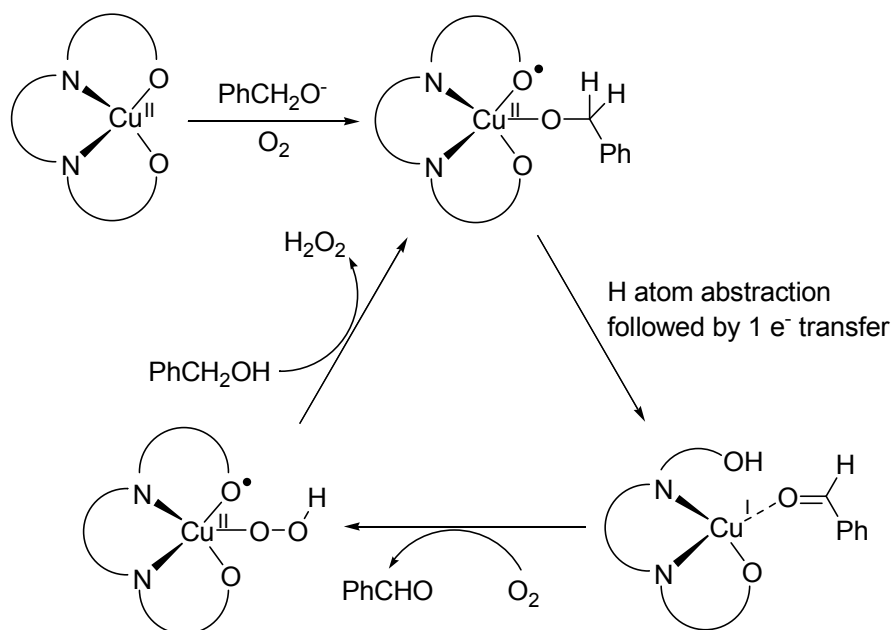
Figure 3-23: Salen type–copper(II) catalysed aerobic oxidation of benzyl alcohol according to *Stack*.[16]

Wieghardt, *Chaudhuri* and co-workers have synthesised the 2,4-di-*tert*-butylphenol derivative ligands **23** and **24**.[19, 87]

23   **24**
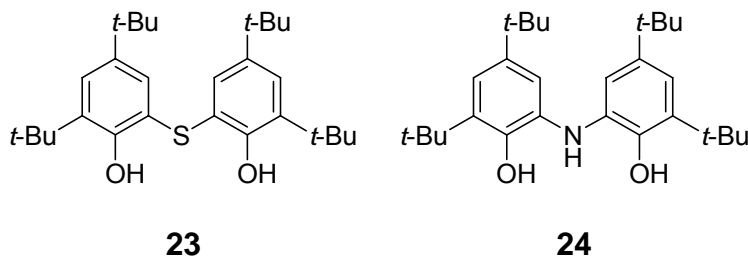
Figure 3-24: 2,4-Di-*tert*-butylphenol derived ligands by *Wieghardt* and *Chaudhuri*.[19, 87]

The copper(II) complexes of ligands **23** and **24** are structural and active models of GOase. In 1998, *Wieghardt* and *Chaudhuri* reported the formation of the dinuclear copper(II) phenoxyl complex **25** by reaction of 2,2'-thiobis(2,4-di-*tert*-butylphenol) ligand **23** with 1 equivalent of copper(I) chloride and 2 equivalents of triethylamine in THF and under a stream of dioxygen (**Scheme 3-8**).[87]
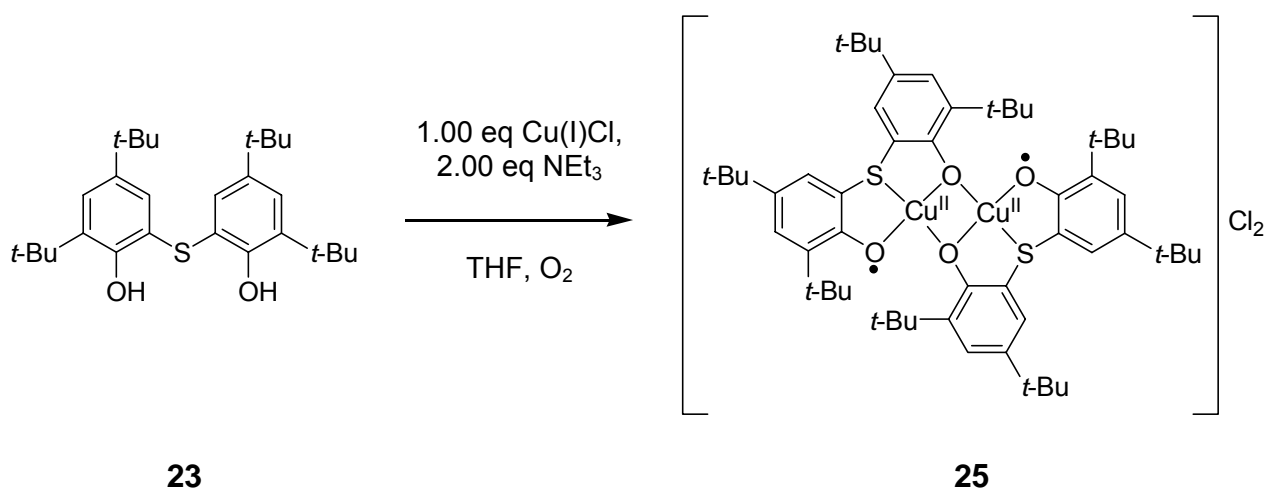
Scheme 3-8: **Formation of dinuclear copper(II) phenoxyl complex 25.**[87]

0.1 Mol% of the dinuclear copper(II) complex **25** generated in situ converted ethanol and benzyl alcohol to their aldehyde, and secondary alcohols to dimeric 1,2-glycols with moderate yields. During the catalytic cycle, hydrogen peroxide is formed by the reduction of dioxygen (**Scheme 3-9**, **Scheme 3-10**).
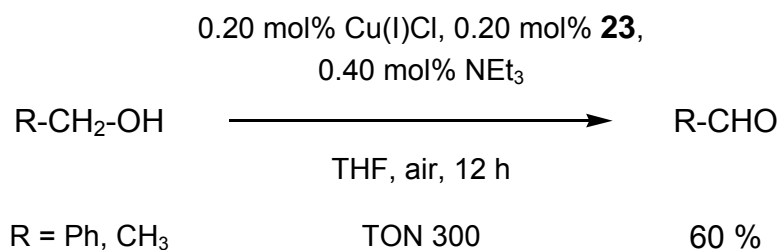
Scheme 3-9: **Aerobic oxidation of primary alcohols with complex 25 generated _in situ_.**
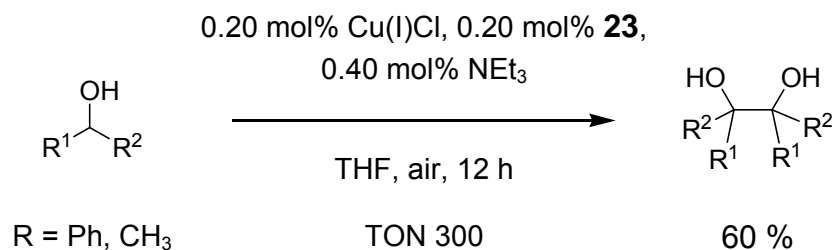
Scheme 3-10: **Aerobic oxidation of secondary alcohols with complex 25 generated _in situ_.**

In 1999, the same group reported the mononuclear copper complex **26** that catalyses the oxidation of primary alcohols (including ethanol, but not methanol) with dioxygen to aldehydes and hydrogen peroxide.[19] The complex was obtained by condensation of the ligand 3,5-di-*tert*-butylcatechol **24** with ammonia and a copper salt. In its active form, the complex contains a tyrosyl radical and a copper(II), similarly to the active centre of GOase. A solution of benzyl alcohol or ethanol in THF containing the complex **26** was stirred in open air at room temperature for 20 hours and gave about 55 % of the corresponding aldehyde, together with hydrogen peroxide. The oxidation can also be carried out in pure alcohol. Kinetic studies have shown the active form to be the mononuclear copper species **26** and kinetic isotope effect studies showed that the α-hydrogen abstraction is the rate determining step of the reaction. With this system, ethanol was oxidised with a turnover frequency of 0.5 s^{-1}. Secondary alcohols are no substrates for catalyst **26**.
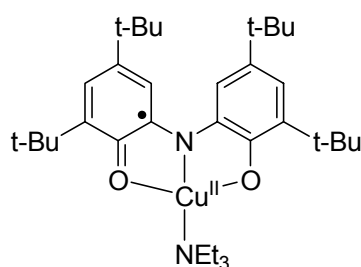
26
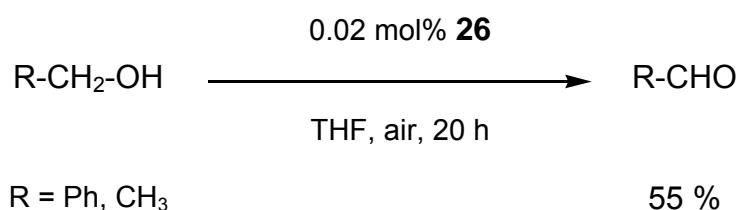
Scheme 3-11: Aerobic oxidation of primary alcohols with complex 26.[19]

A third system reported by *Wieghardt* and *Chaudhuri* is the copper(II) complex **28** of ligand **27** which effectively catalyses the aerobic oxidation of primary alcohols, including methanol, with formation of the corresponding aldehydes and hydrogen peroxide at ambient temperature.[20] Secondary alcohols are no substrates for this system. Ligand **27** was complexed with copper and zinc ions. Up to 5000 turnovers in

50 hours have been achieved in the air oxidation of ethanol by the copper complex, which corresponds to a turnover frequency of 0.03 s^{-1}. In contrast, the zinc analogue is less efficient, and the catalyst stability is inferior (170 turnovers in 24 hours, which corresponds to a turnover frequency of 0.002 s^{-1}). This is the first copper system able to catalyse the aerobic oxidation of methanol. Triethylamine becomes an inhibitor in the oxidation of ethanol when it is used in more than four equivalents because it binds more effectively to the copper complex than the alcohol substrate.

27   **28**
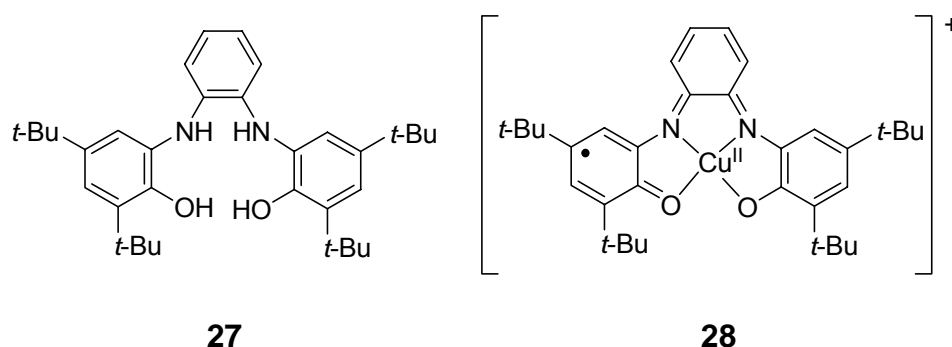
Figure 3-25: **Ligand 27 and the active species 28 in the aerobic oxidation of primary alcohols.**

0.01 mol% **28**,
2 eq NEt$_3$

CH$_3$-CH$_2$-OH $\longrightarrow$ CH$_3$-CHO

CH$_2$Cl$_2$, 1 bar air, 50 h

TOF 0.03 s^{-1}
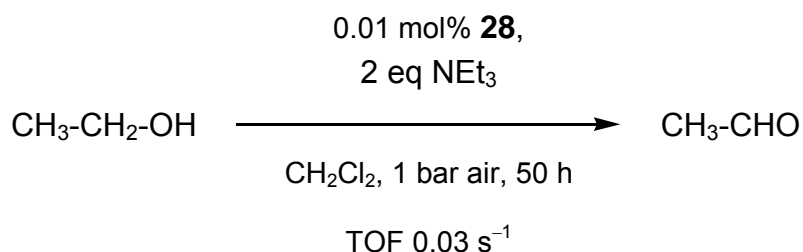
Scheme 3-12: **Aerobic oxidation of ethanol with complex 28.**[20]

Some cyclic voltammetric, EPR and kinetic studies were performed on the complex and, as a result, a mechanistic scheme was proposed for this aerobic oxidation (**Figure 3-26**). In the first step, a rapid equilibrium between the catalyst and one molecule of alcohol is established to give the five-coordinated copper complex **A**. The hydrogen abstraction that follows is most probably the rate determining step, resulting in the formation of a common ketyl radical anion. The resulting intermediate **B** is proposed to undergo a rapid intramolecular one-electron transfer with formation of a bound aldehyde, which is a poor ligand and dissociates. The ultimate electron

acceptor in this step is the *o*-diiminoquinone unit of the doubly protonated ligand. Mechanistically, it is conceivable that the copper(II) ion is the electron acceptor, which is thereby reduced to copper(I), and then intramolecularly re-oxidised to copper(II) to form intermediate **C**. The reduced catalysts react with dioxygen, resulting in hydrogen peroxide and the active catalyst **28**. This reoxidation step most probably is also divided into two successive one-electron-transfer steps, where a coordinated superoxide copper(II) intermediate is formed.
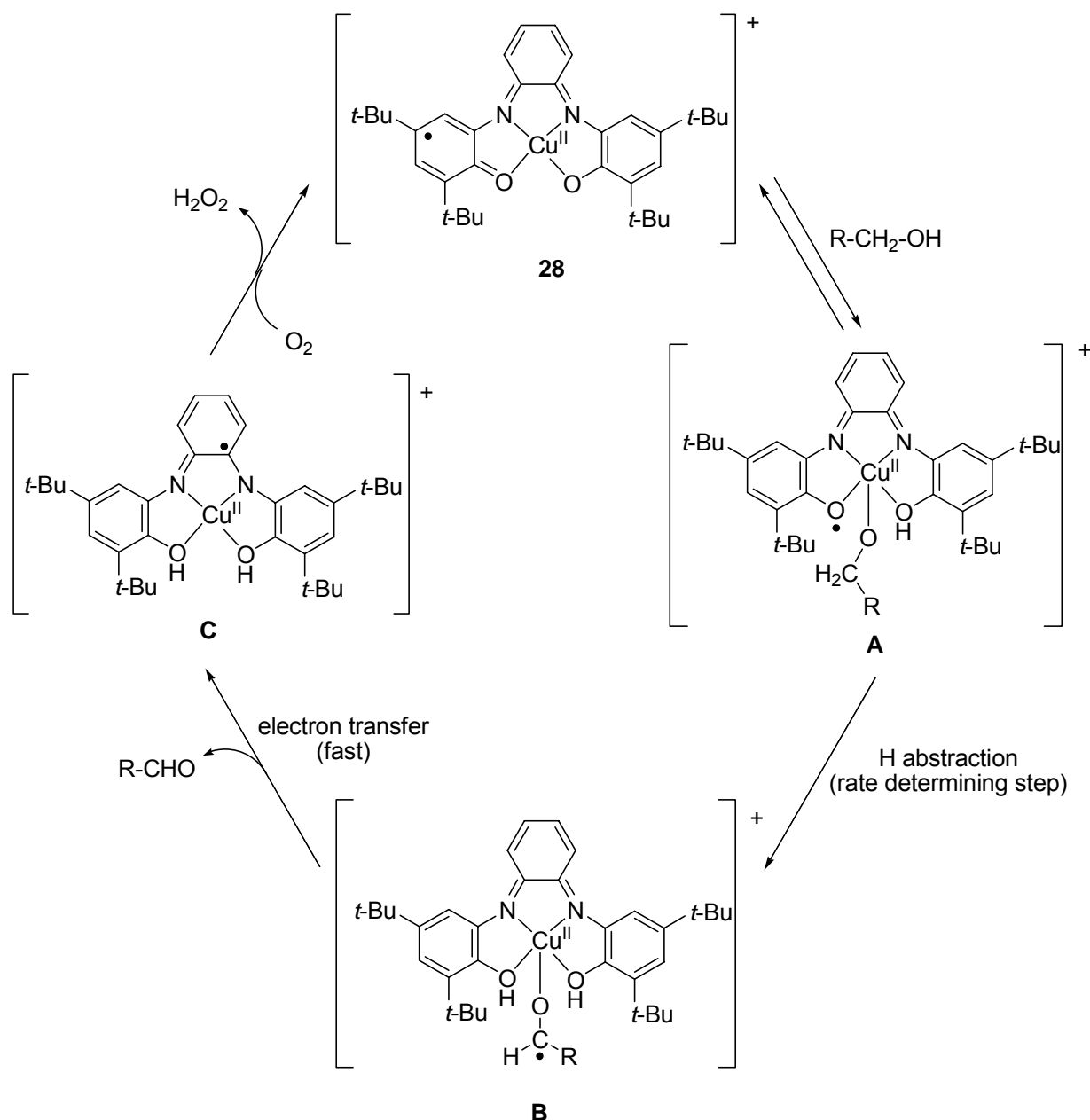
Figure 3-26: **Proposed mechanism for the catalytic oxidation of primary alcohols by dioxygen according to *Wieghardt* and *Chaudhuri*.[20]**

- 43 -

Thomas and co-workers reported the tripodal ligand **29** as a structural and functional model of GOase.[88] Their strategy was based on the differentiation of the two phenolic arms. One of the *tert*-butyl phenolic arms bears an electron-withdrawing nitro group and the other an electron-donating methoxy group.

29   **30**
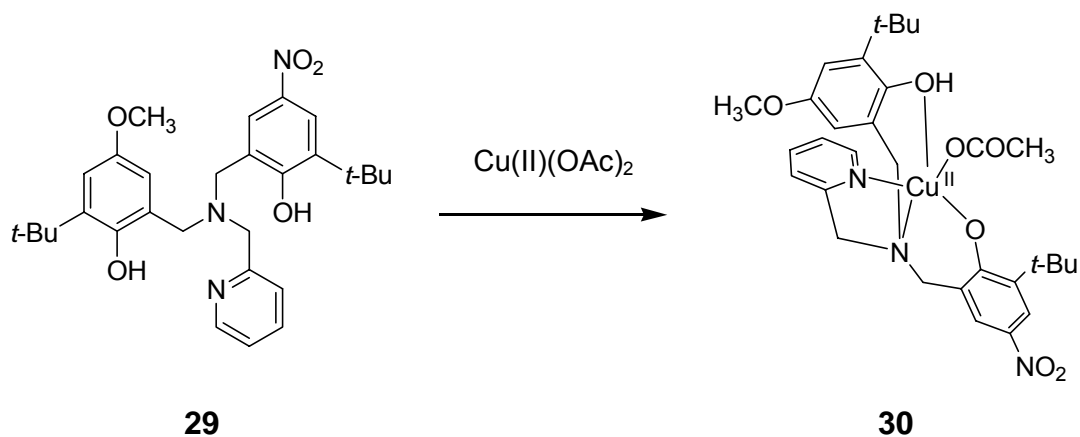
The monomeric copper(II) complex of ligand **29** was obtained. A square pyramidal geometry was identified by X-ray crystallography. It involves a [N$_2$O$_2$] coordination sphere around the copper(II) centre with an acetate molecule in equatorial position. The two different phenolic arms control the geometry of the complex. Indeed, the electron-donating effect of the methoxy group relative to the nitro group makes the deprotonation of the hydroxyl group harder. As a consequence, the weak Cu(II)…OH(Ar) bond occupies the more labile position, that is the axial position. Oxidation of benzyl alcohol to benzaldehyde by the monomeric copper(II) complex was observed in the presence of dioxygen and of a catalytic amount of potassium hydroxide. After 2 days, 220 turnovers were achieved. Again, use of PhCD$_2$OH indicated that the rate-limiting step during oxidation of alcohol is the C–H bond cleavage as for GOase. The use of the electrochemically oxidised form of the complex led to the oxidation of benzyl alcohol with a higher rate (220 turnovers in 2 hours). Unactivated primary alcohols were also oxidased to their corresponding aldehydes, but at a slower rate (80 turnovers in one day for the oxidation of ethanol). Secondary alcohols were not oxidised.

$$\text{R-CH}_2\text{-OH} \xrightarrow[\text{O}_2, \text{ 1 d}]{\textbf{30}, \text{ KOH}} \text{R-CHO}$$

R = Ph   TON 300

R = CH$_3$ TON 80
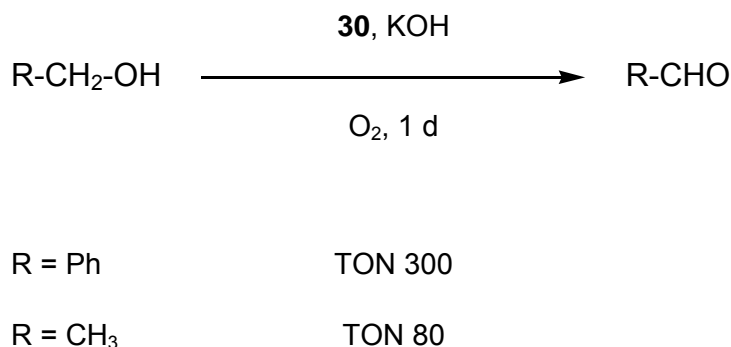
Scheme 3-13:   Aerobic oxidation of ethanol with complex 28.

The sulfonamide ligand **31** was synthesised by *Bulat* and screened for catalytic activity in the aerobic oxidation of benzyl alcohol.[89] Complex **32** was obtained by incubation of ligand **31** with copper(I) chloride and potassium hydroxide in acetonitrile. After addition of methanol and re-crystallisation from methanol, the complex was analysed by crystallography. The X-ray analysis of complex **32** reveals a square planar copper centre. In addition to the tridentate ligand, a methanol molecule is coordinated to the copper ion.

31   **32**
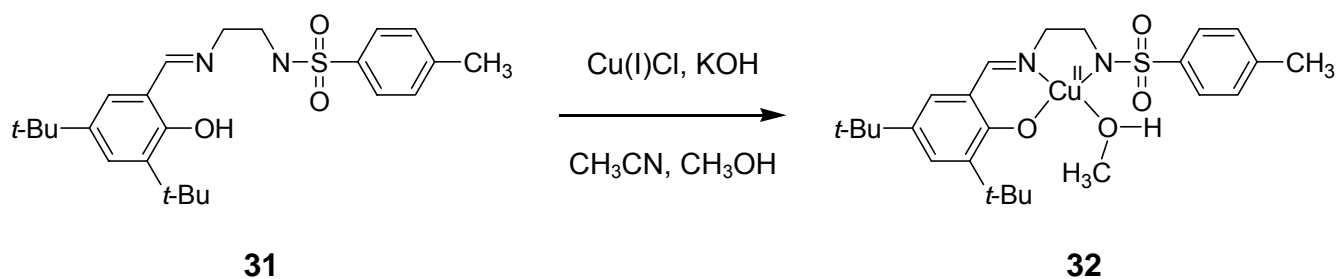
Scheme 3-14:   Complexation of ligand 31 with copper.[89]

Copper complex **32** mediates the catalytic aerobic oxidation of benzyl alcohol with a turnover number of up to 34.
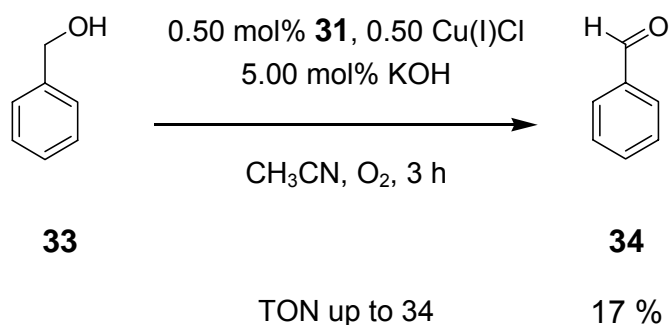
Scheme 3-15: Aerobic oxidation of benzyl alcohol with complex 32.[89]

The activity of the catalyst ceases after 3 hours. The appearance of a brown precipitate was observed in the reaction flask. This precipitation corresponds to the time when the oxidation starts to slow down. The brown solid has been attributed to copper oxide. **Scheme 3-16** shows a plausible pathway leading to the deactivation of the catalyst by formation of a dinuclear copper(II)-trans-μ-peroxo complex.
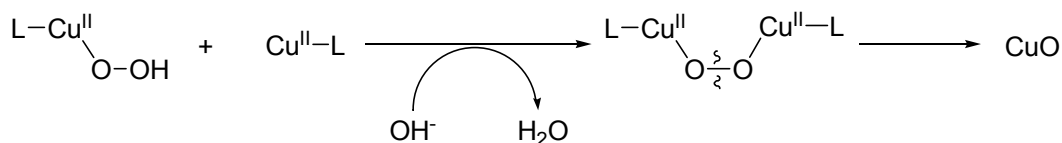
Scheme 3-16: Generation of an unstable dinuclear copper(II)-trans-μ-peroxo compex.

3.4   Peptide libraries as sources of active catalysts

In 1996, *Jacobsen* and co-workers reported the synthesis of combinatorial libraries for the discovery of metal–ligand complexes.[90] The stability and activity of metal complexes are dependent on numerous variables, such as the coordination geometry required by the metal and the steric and electronic characteristics of the ligand. Therefore, combinatorial chemistry provides a powerful approach for the discovery of new coordination compounds. In their approach, *Jacobsen* and co-workers have synthesised ligand libraries with four variable components: two amino acids AA_1 and AA_2, a turn element and various capping ends (**Figure 3-27**). The library, consisting of 12 000 different ligands, was synthesised on amino TentaGel resin, using the standard split-mix technique. The peptide-ligands were encoded with photocleavable tags. The potential for members of this library to form coordination complexes was evaluated by exposing samples of the beads to homogeneous solutions of metal

ions: Ni(II), Fe(III), Cu(II), Pt(IV), Pt(II), Sn(IV), followed by treatment with a stain reagent and observation of the colour of the beads with a microscope. The structure of the ligands on the beads was analysed.
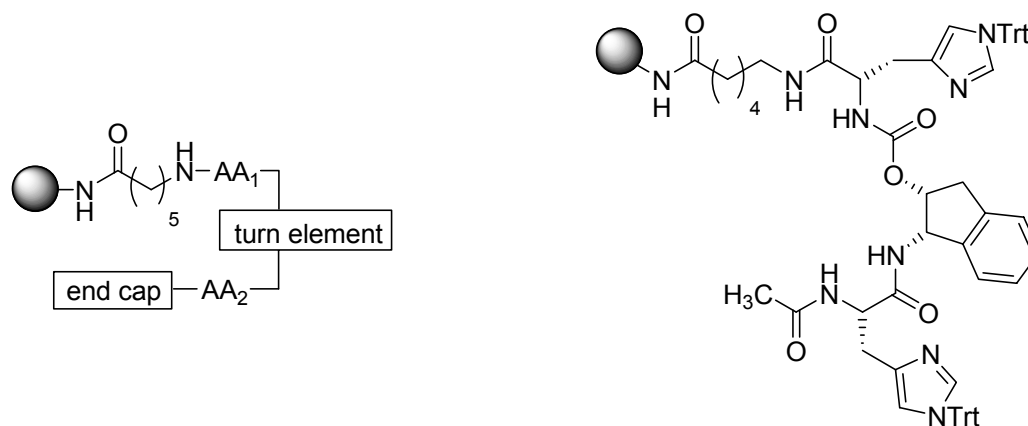
Figure 3-27:   Combinatorial library for the discovery of novel coordination complexes and an example of a structure that binds Ni(II).[90]

The synthesis and sceening of peptide libraries in order to discover active catalysts has been used by *Berkessel* and *Hérault*.[27] In 1999, they reported the synthesis of a 625-member library of undecapeptides and its screening for hydrolase activity (**Figure 3-28**). The library was synthesised on amino TentaGel resin following the split-mix strategy. The ligands were complexed with Lewis acidic transition metals (Cu^{2+}, Zn^{2+}, Fe^{3+}, Co^{3+}, Eu^{3+}, Ce^{4+} or Zr^{4+}). For the screening of the activity of the peptide–ligands, the 3-hydroxyindolyl derivatives **35** or **36** were used. Indeed, when the ester functions are hydrolysed, the resulting indoxyl derivative **37** is oxidised in the aerated solution to afford the turquoise and insoluble indigo dye **38**.

X = *L*-Arg, *L*-His, *L*-Tyr, *L*-Trp or *L*-Ser
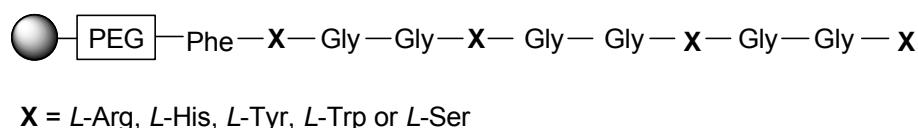
Figure 3-28:   General structure of the undecapeptide library.[27]
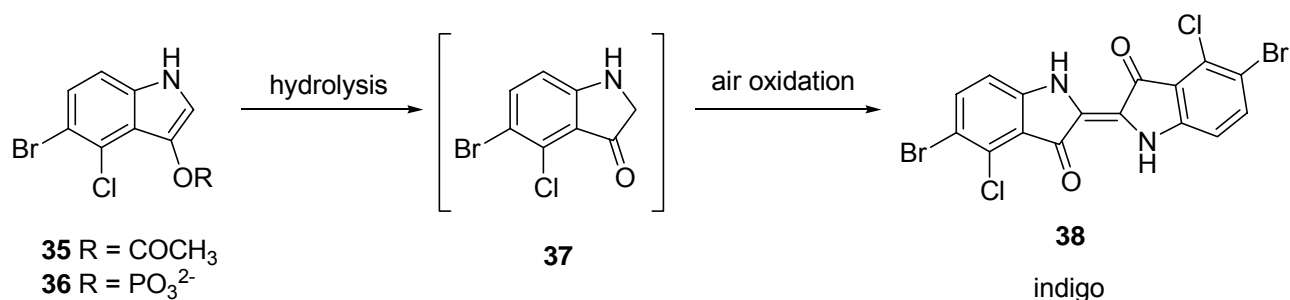
Figure 3-29:   Test substrates for the screening of the library for hydrolysis activity.[27]

The on-bead screening of the library with the test substrates **35** and **36** in the presence of a variety of transition metal ions enabled the identification of members of this library that, in combination with Zr^{4+}, mediate phosphate hydrolysis.
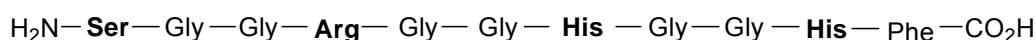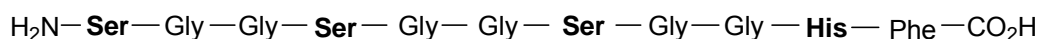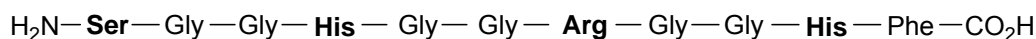
Figure 3-30:   Active sequences found by screening of the undecapeptide library with Zr^{4+} and 36.

As model for the enzyme GOase, the 16-member library described in **Figure 3-31** was synthesised by *Bulat* with the **X_n** positions occupied by histidine or tyrosine.[89] The 16 decapeptides were incubated in a copper salt solution and screened for activity.
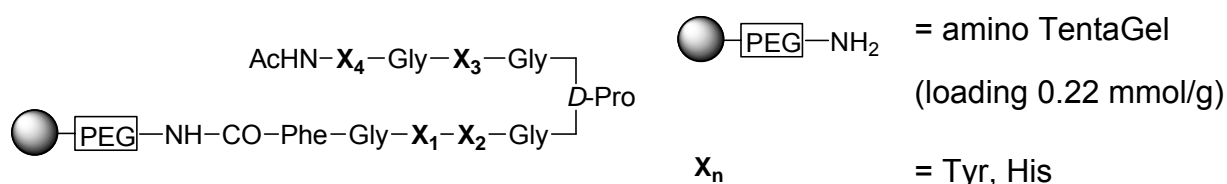
Figure 3-31:   Library of decapeptides as a model for the active site of GOase.[89]

3-Methoxybenzyl alcohol was used as a test substrate to screen the copper(II)–peptide library for catalytic activity. The oxidation product of 3-methoxybenzyl alcohol, 3-methoxybenzaldehyde, absorbs UV-light at λ = 310 nm. 3-Methoxybenzyl

alcohol does not absorb at this wavelength. The library was screened under various reaction conditions: varying the buffer solution and the oxidants (potassium hexacyanoferrate(III), ammonium cerium(IV)nitrate). The copper–peptide complexes were inactive under these conditions. In a last experiment where the TEMPO radical was added in stoichiometric amount to the copper–peptide library, 3-methoxybenzaldehyde was formed (**Figure 3-17**). The best results were obtained for the peptide–ligands where X_1, X_2, X_3, X_4 = Tyr, Tyr, Tyr, Tyr and X_1, X_2, X_3, X_4 = Tyr, His, Tyr, Tyr. However the yield of 3-methoxybenzaldehyde after 22 hours was only 0.4 % for the best peptide sequence (X_1, X_2, X_3, X_4 = Tyr, Tyr, Tyr, Tyr).
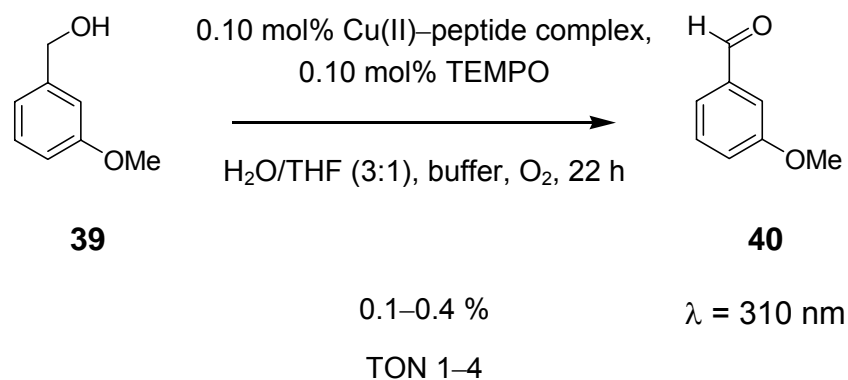
Scheme 3-17: **Aerobic oxidation of 3-methoxybenzyl alcohol with copper(II)–peptide complexes.**[89]

4   Concept

The following work deals with the synthesis of mimics for the enzyme GOase. This choice comes from the interest in developing new environmentally friendly catalysts for the oxidation of primary alcohols to aldehydes. Moreover, structural models for GOase are still needed to get information about the enzyme itself. In a first approach, peptide–copper complexes will be synthesised as models for GOase. In a second approach, a solid-supported low molecular weight copper complex will be synthesised as a model for the enzyme.

4.1   Peptidic model for galactose oxidase

The active site of GOase is a copper ion coordinated by four amino acids.[15] As a consequence, peptides complexed to a copper ion appear to be good structural models for the active site of the enzyme.

The 16-membered peptide library 1 (**Figure 4-1**) was reported by *Bulat*.[89] The peptide–copper complexes have shown catalytic activity only when the radical TEMPO was added in the reaction mixture. The work of *Whittaker* and *Fukuzumi* has shown that the Tyr–Cys cross-link is necessary for the catalytic activity of the enzyme.[75] Moreover, *Stack* reported that copper complexes with phenol ligands possessing a thioether substituent in *ortho* position to the hydroxyl function are more efficient in catalysis than the equivalent copper–phenol complexes without thioether substituent.[16]
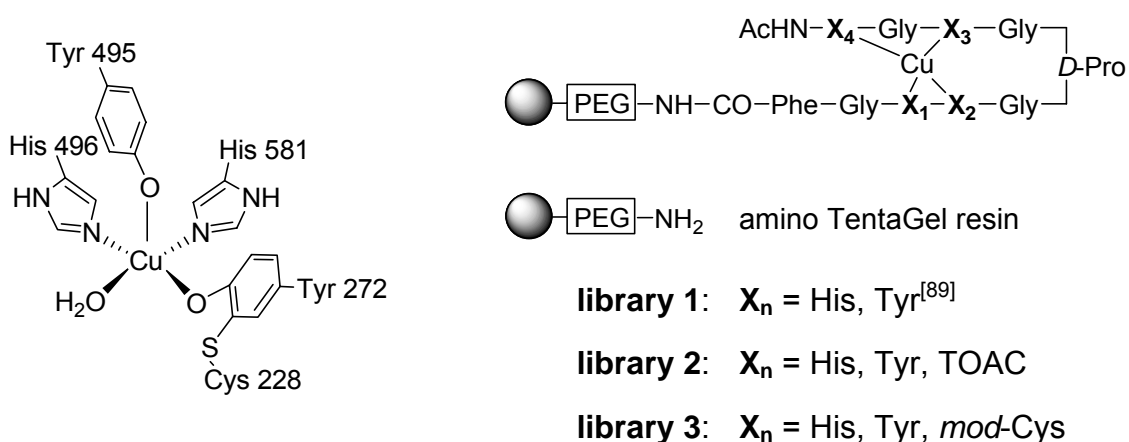
Figure 4-1:   **Active site of GOase and libraries of peptide–copper complexes designed to mimic the active site of the enzyme.**

Taking these results into account, a third amino acid, in addition to histidine and tyrosine, should be added to the variable positions of the peptide library 1 in order to find active catalysts in the library. TOAC **41**, which is a radical containing amino acid derivative of TEMPO, has been chosen. It is known from the work of *Semmelhack* that TEMPO, in combination with a copper ion, mediates the aerobic oxidation of alcohols.[91] This result, in addition to the work of *Bulat*, makes TOAC **41** a good candidate as a mimic for the Tyr–Cys cross-link.

Another amino acid has been designed to mimic the Tyr–Cys cross-link found at the active site of GOase. The modified cysteine (*mod*-Cys, **42**) possesses a phenol moiety with a thioether linkage in ortho position to the hydroxyl function. The second ortho position, as well as the para position, are occupied by *tert*-butyl substituents. Indeed, *Stack* and co-workers have shown that in GOase models, a complete *ortho,para*-substitution of the phenolate ring is necessary to prevent radical-ligand coupling.[86]
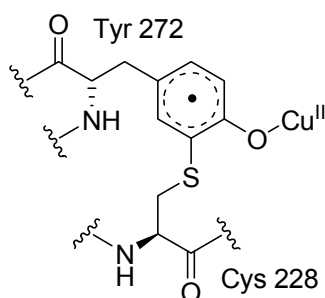
Figure 4-2:   **Tyr–Cys cross-link at the active site of GOase.**
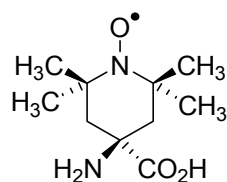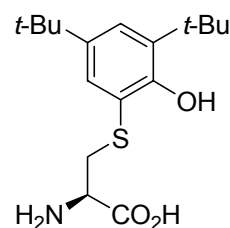
TOAC, **41**   *mod*-Cys, **42**

Figure 4-3:   **Amino acids as models for the Tyr–Cys cross-link found at the active site of GOase.**

The 81-member peptide libraries 2 and 3 (**Figure 4-1**) have been designed to mimic the enzyme GOase. The four X_n positions are occupied by the functional amino acids, histidine, tyrosine and TOAC **41** or *mod*-Cys **42**. The first amino acid linked to the resin is phenylalanine. It is intented to prevent the formation of diketopiperazine when attaching the next amino acid.[58] Glycines have been included to provide the desired shape to the peptide and the sequence *D*-Pro-Gly has been introduced to make a turn in the peptide–ligand so that it could complex around a copper ion.[92] Finally, the last amino acid will be acetylated to avoid complexation at the terminal amino group.

Modeling studies, using MacroModel, of Ac-NH-**His$_1$**-Gly-**Tyr**-Gly-*D*-Pro-Gly-**His$_2$**-**TOAC**-Gly-Phe-COOMe with copper(II) showed a His$_1$–copper distance of 2.3 ± 0.2 Å, a Tyr–copper distance of 5.5 ± 2.7 Å to minimal 2.8 Å, a His$_2$–copper distance of 2.5 ± 0.3 Å and a TOAC–copper distance of 2.6 ± 0.3 Å. The corresponding distances in the enzyme are 1.94 Å for Tyr-Cys–copper, 2.11 Å and 2.15 Å for His–copper and 2.69 Å for the axial Tyr–copper. This model shows that the peptide-ligands should be able to bind a copper ion. In the model, the tyrosine is more distant from the copper ion than the other amino acid residues, indicating a preference for an axial position.
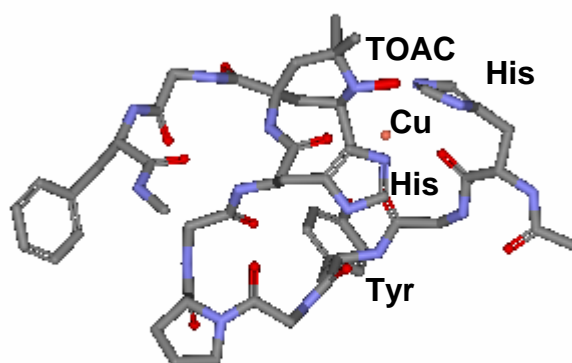
Figure 4-4: **Calculated model of the copper complex of the peptide-ligand Ac-NH-His$_1$-Gly-Tyr-Gly-*D*-Pro-Gly-His$_2$-TOAC-Gly-Phe-COOMe (Monte-Carlo calculation method, gas phase, MMFFs force field).**

The split-mix method, in association with the *IRORI* "directed synthesis" system, is the method of choice for the synthesis of small size libraries. As a solid support, amino TentaGel was chosen because of its swelling properties. Indeed, this polystyrene resin with polyethylene glycol spacer swells in DMF, necessary for peptide synthesis, as well as in water, which is necessary for the catalysis. The libraries will be synthesised in *IRORI* MikroKans. These Kans can contain up to 30 mg of resin, which is a reasonable quantity that allows screening for catalytic activity. The well-established Fmoc/PyBOP strategy will be used for peptide synthesis.

For peptide synthesis, the hydroxyl function of tyrosine as well as the amine function of histidine must be protected in order to avoid side reactions at these groups. The protecting groups *tert*-butyl and trityl were chosen for the protection of tyrosine and histidine, respectively, because of their stability under the conditions of the peptide synthesis. They can be removed under acidic conditions after the acetylation step.

The amino functions of the two unnatural amino acids should be protected with Fmoc in order to allow quantification of the peptide couplings. The radical containing Fmoc-TOAC-OH **43** is known from the litarature and will be synthesised according to the synthesis described by *Dulog* and co-workers.[93]
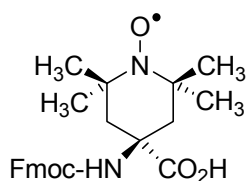
43

As shown by the work of *Bulat*, the hydroxyl function of the Fmoc-*mod*-Cys-OH amino acid **44** needs to be protected. Indeed, it proved impossible to incorporate **44** into a peptide chain because of the formation of the lactone **45** (**Scheme 4-1**).[89]

2.5 eq PyBOP, 3 % NMM

CH$_2$Cl$_2$

44 27 % **45**
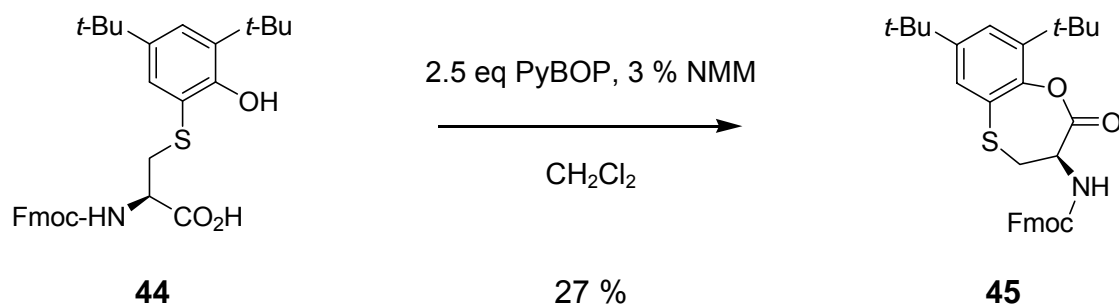
Scheme 4-1: Lactonisation of the amino acid 44 under peptide coupling conditions.[89]

The protecting groups methoxymethyl (MOM) and allyl have been chosen for the protection of the phenolic function since they are both stable under basic conditions (see later).

Two strategies were planned for the synthesis of the enantiomerically pure Fmoc-*mod*-Cys(PG)-OH (**Figure 4-5** and **Figure 4-6**). Both strategies are convergent syntheses and start from an enantiomerically pure amino acid. If racemisation is avoided, the synthesis should lead to an enantiomerically pure product.

In the first strategy, *L*-cystine is used as a starting material (**Figure 4-5**). Its coupling with the MOM-protected bromo-phenol **48** by bromine/lithium exchange and electrophilic substitution should give the Boc-*mod*-Cys(MOM)-OH **47**. The Boc protecting group has been chosen for the protection of the amino function since it is stable under the basic conditions necessary for the coupling of compounds **48** and **49**. The carboxylic acid function of the cystine derivative should be protected by methylation to avoid deprotonation by the organolithium. The organolithium could then react either at the carbonyl function of **49** to form an alcohol or at the disulfide to form compound **47**. The MOM-protected bromo-phenol **48** would be obtained from the commercially available 2,4-di-*tert*-butylphenol **51** by bromination and protection of the hydroxyl function with the MOM protecting group. The bromination in ortho position to the hydroxyl function is favoured due to the directing effect of the OH group and the steric constraints of the 2 *tert*-butyl groups. Finally, the target compound **46** would be obtained by removal of the Boc protecting group of the amino acid **47**, followed by Fmoc protection of the free amino function. The removal of the Boc should be possible without affecting the MOM protecting group. Indeed, while

MOM is cleaved under aqueous acidic conditions (HCl), Boc should be cleaved under anhydrous acidic conditions (TFA). Therefore, treatment of the amino acid **47** with TFA in dry dichloromethane should lead to the removal of the Boc without cleavage of the MOM protecting group.
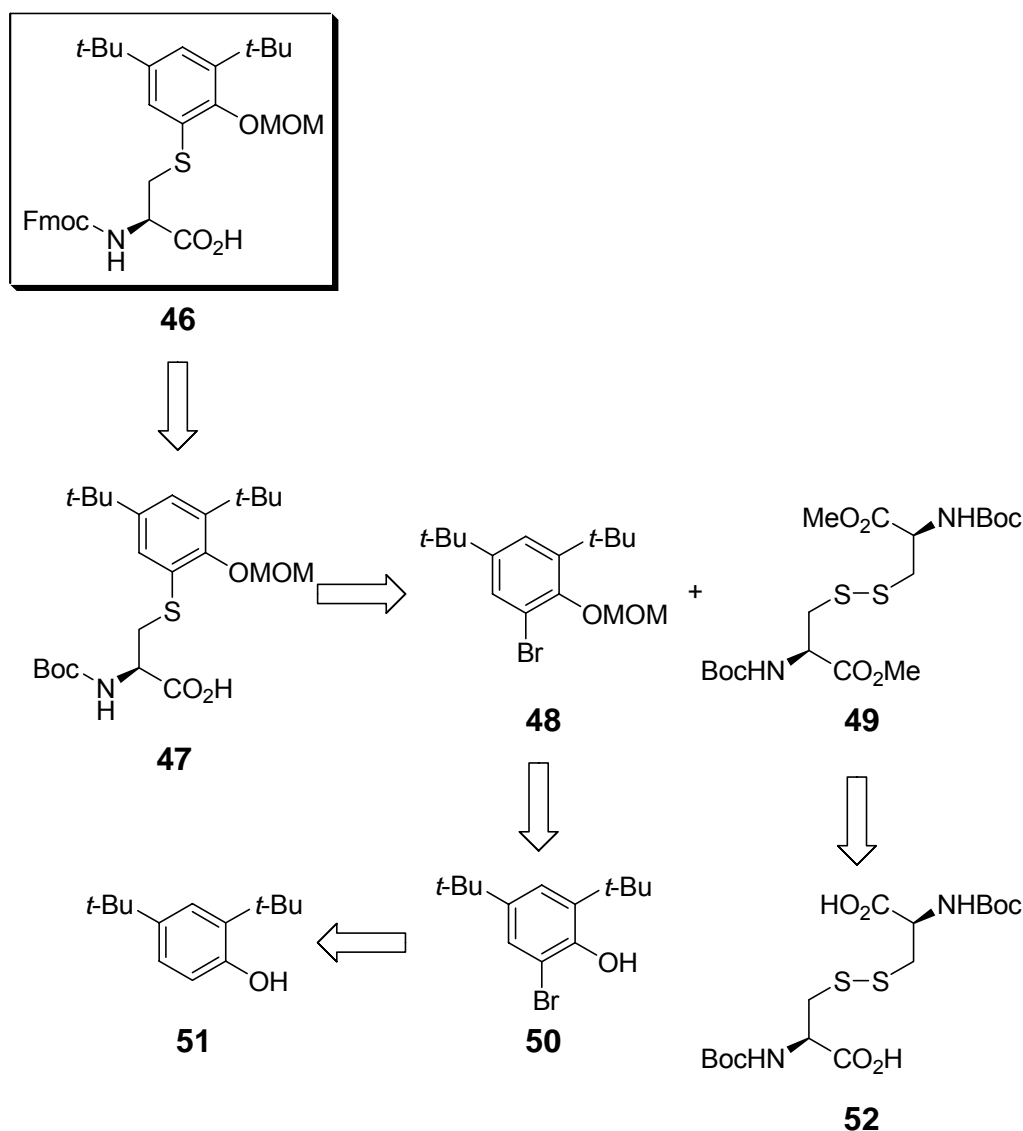
Figure 4-5: **Retro-synthesis of the *N*-Fmoc-protected *mod*-Cys amino acid. Strategy using *L*-cystine as an enantiomerically pure starting material.**

In the second strategy, *L*-serine is used as a starting material (**Figure 4-6**). Serine lactones are widely used as intermediates in the enantioselective synthesis of β-substituted alanines, giving rise to many unnatural amino acids and other chiral building blocks.[94] *Vederas* and co-workers opened serine lactones with various nucleophiles, including amines, thiols, halogens, and a variety of organometallic

reagents.[95] The ring opening of the lactone **57** or **58** by the thiol **55** or **56** in basic conditions would lead to the *N*-protected *O*-protected *mod*-Cys **47**, **53** and **54**.

Starting from the *N*-protected-*L*-serine **59** or **60**, the *N*-protected-*L*-serine-β-lactone **57** and **58** could be formed by a *Mitsunobu* reaction.[95, 96] To avoid polymerisation during the synthesis of the serine lactone **57** and **58**, as well as for the reaction of the thiol **55** or **56** with the lactone **57** or **58**, the amine function of the serine must be protected. Since the ring opening of the lactone will be carried out under basic conditions, the amine function has to be protected with a base-stable protecting group. The acid-labile Boc protecting group was chosen. The Cbz protecting group was also candidate because it is cleavable by catalytic hydrogenation.

The thiols **55** and **56** can be synthesised from 2,4-di-*tert*-butylphenol **51**. In a first step, the unprotected thiol **64** can be synthesised from 2,4-di-tert-butylphenol **51** according to the procedure described by *Pastor* using sulphur monochloride.[97] Then the hydroxyl function of compound **64** needs to be protected. The thiyl function is more reactive than the hydroxyl function. Thus the strategy consists in protecting the thiol by formation of the disulfide **63**, followed by protecting the hydroxyl function and reducing the disulfide back to the thiol. The thiol **64** could be oxidised to the disulfide **63** by iodine or bromine. Protection of the hydroxyl function by MOM or allyl could be performed in the standard conditions using MOMCl or allyl chloride. The reduction of the disulfides **61** and **62** to the thiols **55** and **56** could be carried out with sodium borohydride.

Finally, the coupling of the lactone **57** or **58** with the thiol **55** or **56** should be possible after deprotonation of the thiol. The last step of the synthesis should be the selective cleavage of the *N*-protecting group and protection of the free amino function by Fmoc, to give the Fmoc-*mod*-Cys-OH.
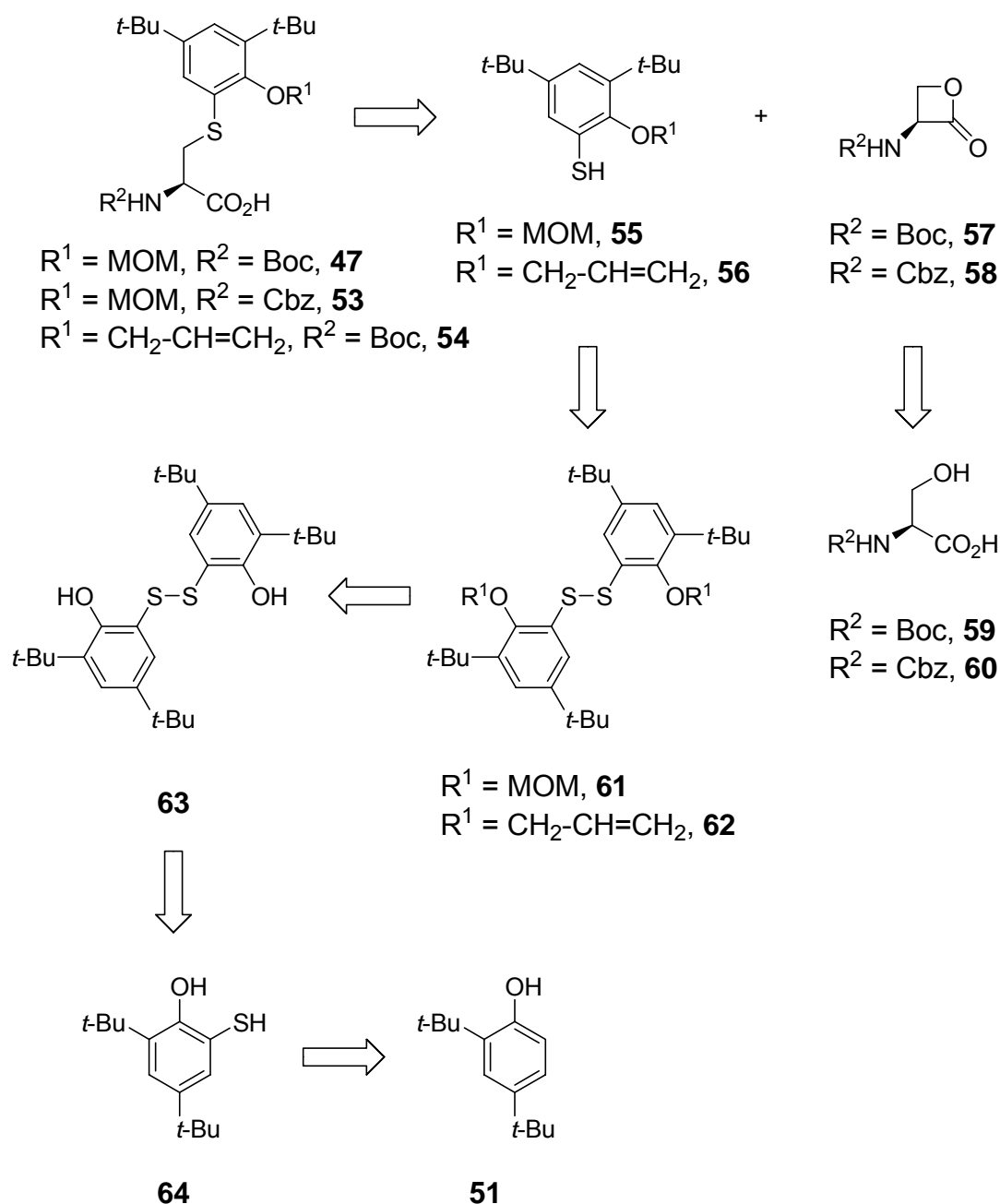
Figure 4-6: Retro-synthesis of the *N*-Fmoc-protected *mod*-Cys amino acid. Strategy using *L*-serine as an enantiomerically pure starting material.

After the synthesis of the library, the peptide-ligand should be complexed with copper. Finally, the peptide–copper complexes should be screened in the catalytic aerobic oxidation of primary alcohols. The oxidation of 3-methoxybenzyl alcohol to 3-methoxybenzaldehyde could be used as a test reaction (**Scheme 4-2**). Indeed, 3-methoxybenzaldehyde absorbs in UV at $\lambda = 310$ nm where 3-methoxybenzyl alcohol does not absorb. Therefore the active catalysts could be identified following the absorbance of the reaction mixture at $\lambda = 310$ nm. This test reaction has already

been used by *Branchaud* and co-workers,[64] as well as by *Kosman* and co-workers.[98]

absorption at $\lambda = 310$ nm

39   **40**
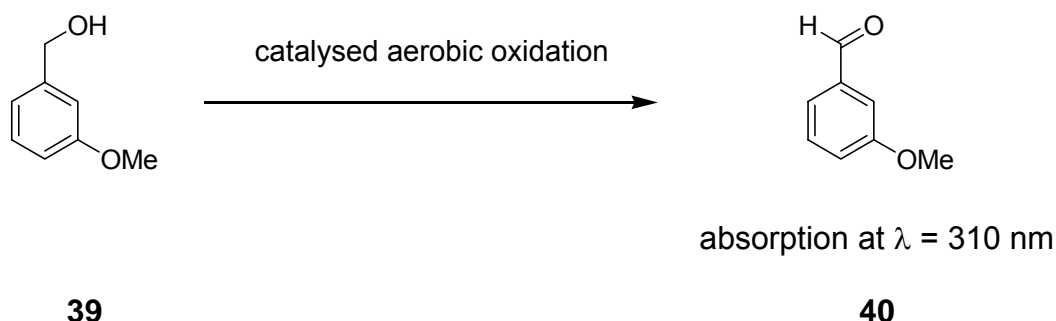
Scheme 4-2: Test reaction for the screening of the library of peptide–copper complexes.[64, 98]

4.2 Solid-supported low molecular weight model for galactose oxidase

In another part of this work, a low molecular weight ligand should be synthesised on solid support, and its catalytic activity in the aerobic oxidation of primary alcohols should be compared to the activity of its soluble counterpart.

Copper complex **32** has been synthesised by *Bulat*.[89] This copper complex mediated the catalytic aerobic oxidation of benzyl alcohol. However after 3 hours the deactivation of the catalyst was observed. As an alternative, the solid-supported copper complex **65** should be synthesised. It was thought that the steric environment caused by the solid support could prevent the degradation of the catalyst. As a solid support, a polystyrene resin could be used.

32   **65**
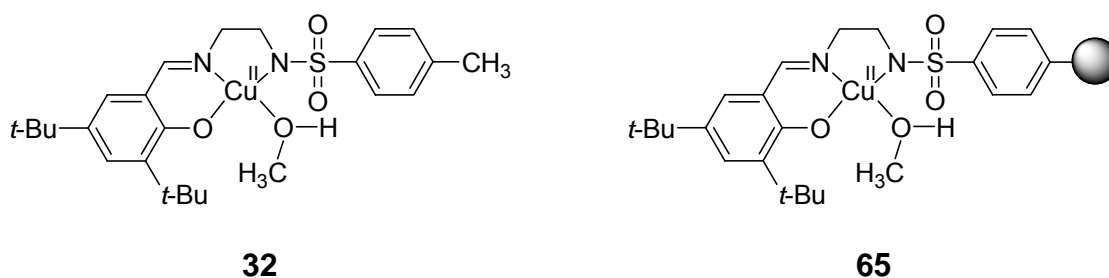
Ligand **66** should be synthesised in 2 steps from chlorosulfonated polystyrene resin (PS-TsCl) **69** (**Figure 4-7**). This synthesis uses the advantage of solid-phase

synthesis, i.e. employing an excess of reagent, the reaction should be driven to completion. The reaction of the PS-TsCl resin **69** with ethylenediamine should give rise to the monosulfonated diamine **67**. The reaction of compound **67** with 2,4-di-*tert*-butyl-salicylaldehyde should result in ligand **66**.
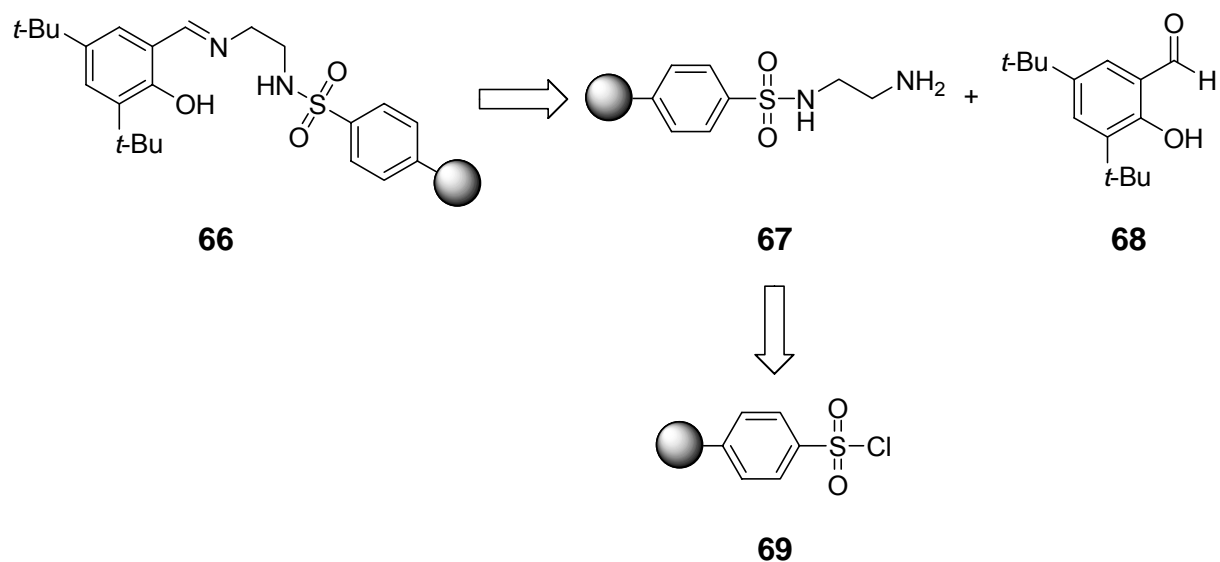
Figure 4-7: Retro-synthesis of the solid-supported low molecular weight ligand 66.

The activity of ligand **66** in the copper catalysed aerobic oxidation of benzyl alcohol and 3-methoxybenzyl alcohol should be compared to the activity of its soluble counterpart **31**.

5   Results

5.1   Synthesis of the unnatural *mod*-Cys amino acid: model for the Tyr-Cys cross-link of GOase

The first strategy towards the synthesis of the unnatural PG^2-*L-mod*-Cys(PG^1)-OH amino acid consists of the synthesis of the *O*-protected bromo-phenol **48**. After the bromo/lithium exchange occurred, the organolithium should react with the *O,N*-protected cystine **49** and give Boc-*L-mod*-Cys(MOM)-OMe **70**.

The second strategy is inspired by the work of *Vederas* and co-workers and consists in the ring opening of a *N*-protected *L*-serine-β-lactone by a *O*-protected thiophenol.[95]

Both strategies lead directly to enantiomerically pure modified *L*-cysteines.
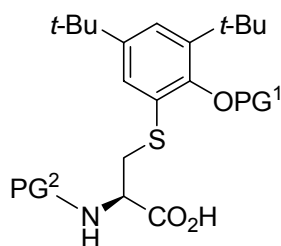
PG^2-*L-mod*-Cys(PG^1)-OH

Figure 5-1: General structure of the protected unnatural *mod*-Cys amino acid.

5.1.1   First pathway towards the synthesis of the unnatural mod-Cys amino acid

5.1.1.1 Synthesis of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene **48**

The first route started with the commercially available 2,4-di-*tert*-butylphenol **51** which was converted to the *O*-protected bromo phenol **48** in two steps (**Scheme 5-1**). Bromination of **51** gave the *ortho*-bromophenol **50** in 92 % yield.[99] The latter was protected by a MOM protecting group using MOMCl under basic conditions in 82 % yield.[100]
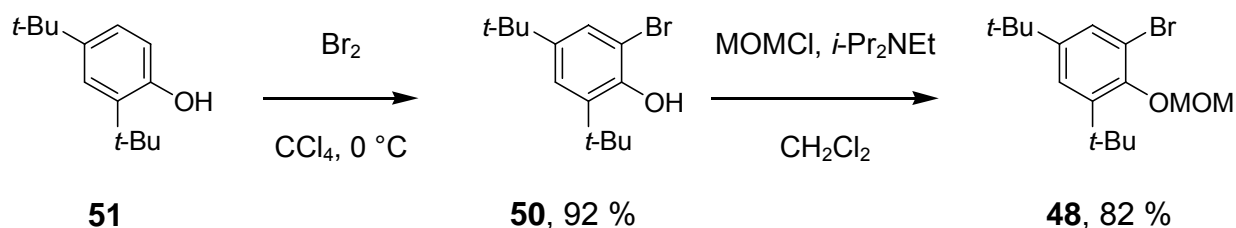
Scheme 5-1: Synthesis of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene 48 from 2,4-di-*tert*-butylphenol 51.[99, 100]

5.1.1.2 Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene 48 and reaction with various electrophiles

In order to avoid deprotonation by the organolithium, the acid function of the commercially available *N,N'*-bis-(*tert*-butyloxycarbonyl)-*L*-cystine **52** was converted to the methylester **49** in a reaction with diazomethane (**Scheme 5-2**).[101]
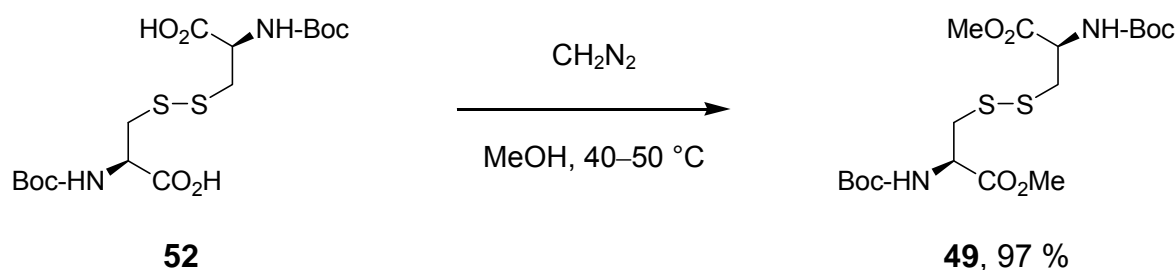
Scheme 5-2: Methylation of *N,N'*-bis-(*tert*-butyloxycarbonyl)-*L*-cystine 52.[101]

A bromine/lithium exchange was carried out on the *O*-protected bromophenol **48** using *n*-butyl lithium and the product was reacted in situ with *N,N'*-bis-(*tert*-butyloxycarbonyl)-*O,O'*-bis-(methyl ester)-*L*-cystine **49**.[102, 103] The resulting compound was the MOM-protected 2,4-di-*tert*-butylphenol **71** and not the expected cysteine derivative **70** (**Scheme 5-3**).

48

1) *n*-BuLi, THF, −78 °C

//

2) **49**, r.t.

70

1) *n*-BuLi, THF, −78° C

2) **49**, r.t.

71, 94 %
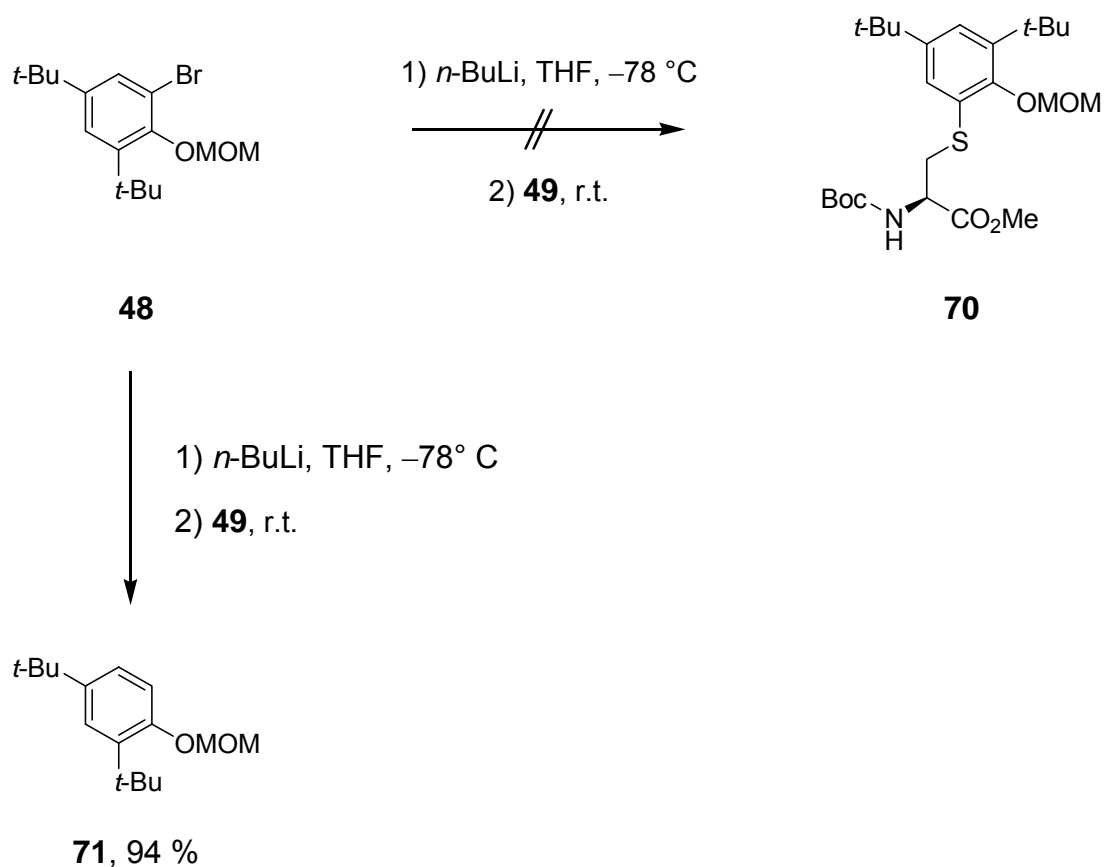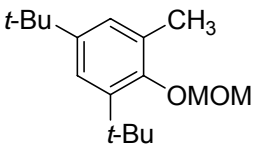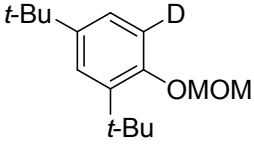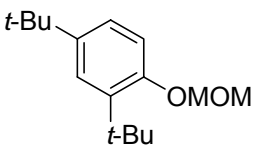
Scheme 5-3: Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene 48.[102, 103]

In order to check that the organolithium was indeed formed, the *O*-protected bromophenol **48** was lithiated and the product was reacted in situ with the electrophiles: methyl iodide and deutero methanol (**Scheme 5-4**, **Figure 5-2**). The expected products **72** and **73** were isolated. However the organolithium did not react with the disulfide **49**. After hydrolysis, the product **71** was isolated.

48

1) *n*-BuLi, THF, −78 °C

2) E⁺, RT
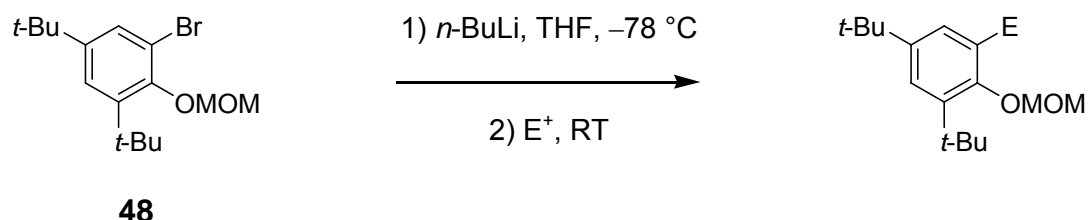
Scheme 5-4: Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene 48 and reaction with various electrophiles.[102, 103]

electrophile (E$^+$)	isolated product	yield
MeI	*t*-Bu, CH$_3$, OMOM, *t*-Bu **72**	90 %
MeOD	*t*-Bu, D, OMOM, *t*-Bu **73**	99 %
49	*t*-Bu, OMOM, *t*-Bu **71**	94 %

Figure 5-2: Electrophiles used and products obtained in the reaction with the organolithium formed from 48.

The reaction of the organolithium with the dissulfide **49** as a nucleophile was not possible. Therefore the first pathway towards the synthesis of the unnatural *mod*-Cys amino acid was abandoned.

5.1.2 Second path towards the synthesis of the unnatural *mod*-Cys amino acid

For the synthesis of the modified cysteine *mod*-Cys following the second strategy, two building blocks were prepared: the *O*-protected 3,5-di-*tert*-butyl-2-benzenethiol [3,5-di-*tert*-butyl-2-(methoxymethoxy)benzenethiol **55** or 3,5-di-*tert*-butyl-2-(allyloxy)-benzenethiol **56**] and the *N*-protected *L*-serine-β-lactone [*N*-(*tert*-butyloxycarbonyl)-*L*-serine-β-lactone **57** or *N*-(benzyloxycarbonyl)-*L*-serine-β-lactone **58**].

5.1.2.1 Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(hydroxy)phenyl]disulfane **63**

The *O*-protected 3,5-di-*tert*-butyl-2-benzenethiols **55** and **56** were synthesised in five steps from 2,4-di-*tert*-butylphenol **51**. According to the procedure described by

Pastor,[97] reaction of **51** with sulphur monochloride gave the trithiobisphenol **74**. The latter was reduced with zinc under acidic conditions to give the thiol **64** in 42 % yield over two steps. For the selective protection of the hydroxyl function of the thiol **64**, the more reactive thiyl function had to be protected first. A thiyl function can easily be protected by oxidation to its disulfide. The thiol **64** was oxidised with molecular bromine on hydrated silica gel support to the disulfide **63** in 91 % yield, without over-oxidation to the corresponding sulfone or sulfoxide.[104]
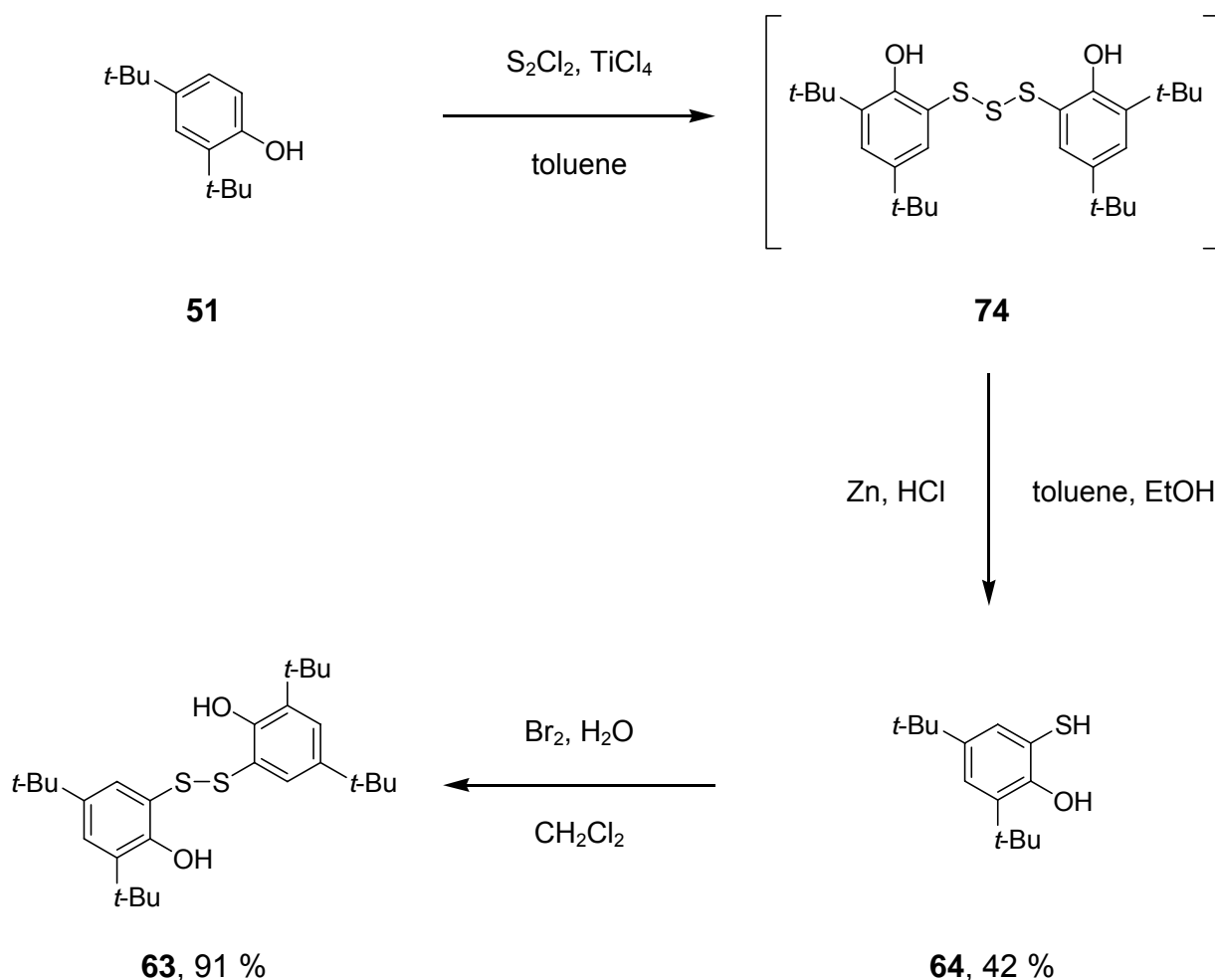
Scheme 5-5: Synthesis of 2,4-di-*tert*-butyl-6-mercaptophenol 64 and protection of the thiyl function by formation of 1,1'-bis[3,5-di-*tert*-butyl-2-(hydroxy)phenyl] disulfane 63.[97, 104]

The disulfide **63** was recrystallised from acetonitrile. The crystals were suitable for X-ray analysis.
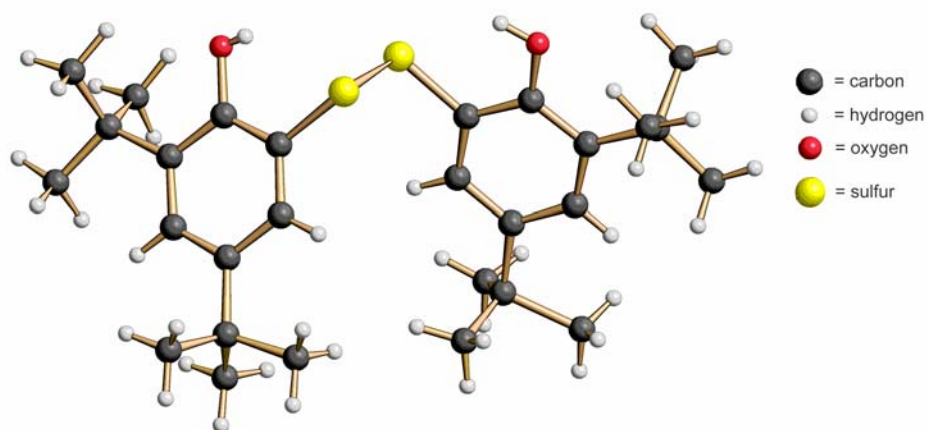
Figure 5-3: X-ray structure of disulfide 63.

5.1.2.2 Protection of the hydroxy functions of 1,1'-bis-[3,5-di-*tert*-butyl-2-(hydroxy)-phenyl]disulfane **63**

The hydroxyl groups of the disulfide **63** were protected by reaction with allylchloride under basic conditions to give the disulfide **62** in 66 % yield.
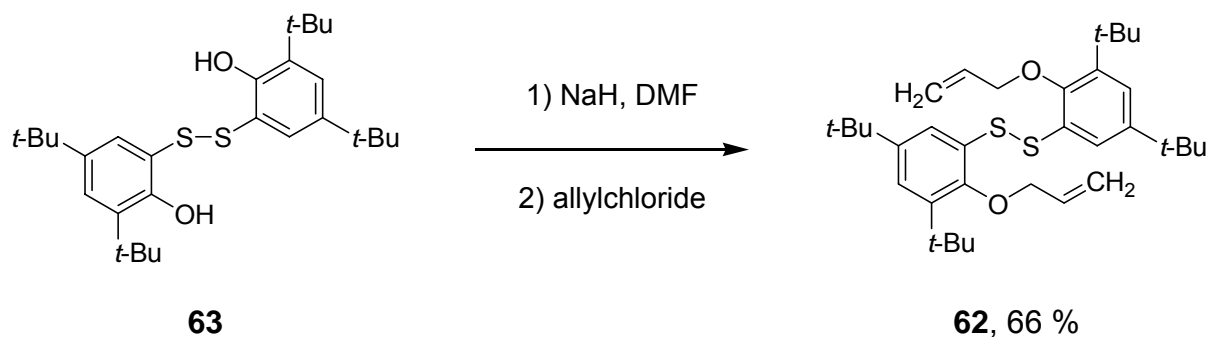
Scheme 5-6: Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(allyloxy)phenyl]disulfane 62.[105]

In the same way, reaction of the disulfide **63** with MOMCl in the presence of *Hünig* base gave the disulfide **61** in 87 % yield.
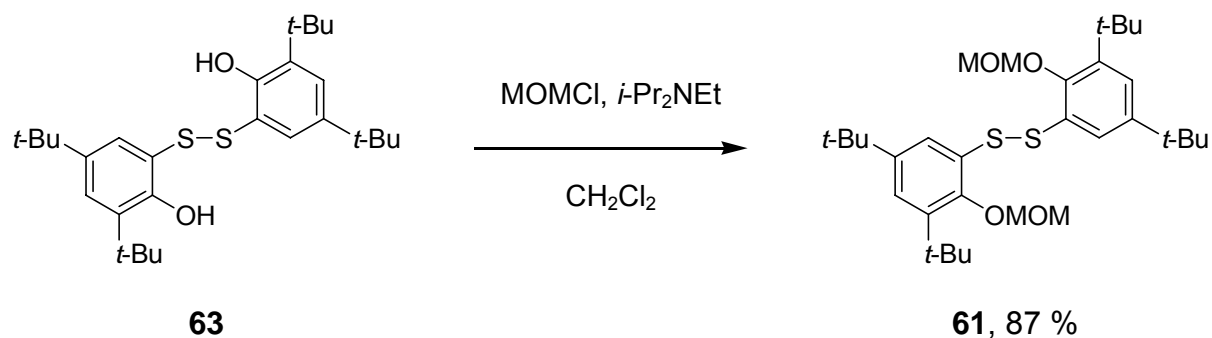
Scheme 5-7: **Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(methoxymethoxy)phenyl]-disulfane 61.[100]**

5.1.2.3 Reduction of the disulfides 61 and 62 to the corresponding thiols

By treatment with sodium borohydride, the disulfide **62** was converted quantitatively to the *O*-protected thiol **56**.[106] The thiol **56** could be analysed by NMR but proved to be unstable and cyclised rapidly to the product **75**. The cyclisation occurs in the presence of water during the work up. If no water is present, the product is re-oxidised to the disulfide **62** by dioxygen (**Scheme 5-8**).
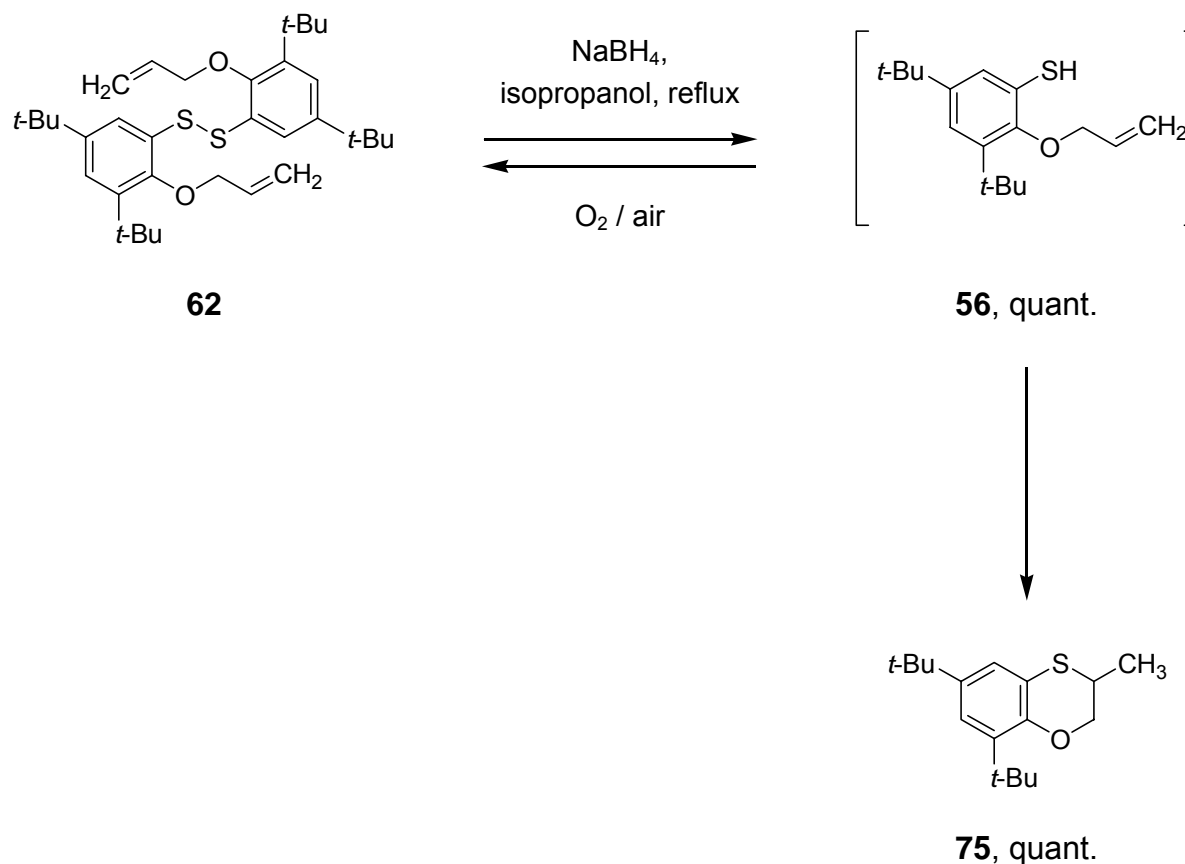
Scheme 5-8: **Reduction of 1,1'-bis[3,5-di-*tert*-butyl-2-(allyloxy)phenyl]disulfane 62 with sodium borohydride.[106]**

When the thiol **56** was stored under argon, it proved to be stable. Nevertheless, this reaction path involving thiol **56** was discarded because of the necessity to obtain the end product *mod*-Cys in a multigram scale.

The reduction of the disulfide **61** with sodium borohydride afforded the *O*-protected thiol **55** in 89 % yield. After purification by column chromatography, product **55** proved to be more stable than its allyloxy equivalent **56** and could even be crystallised from acetonitrile. The crystals were suitable for X-ray analysis which confirmed the structure of the product (**Figure 5-4**).
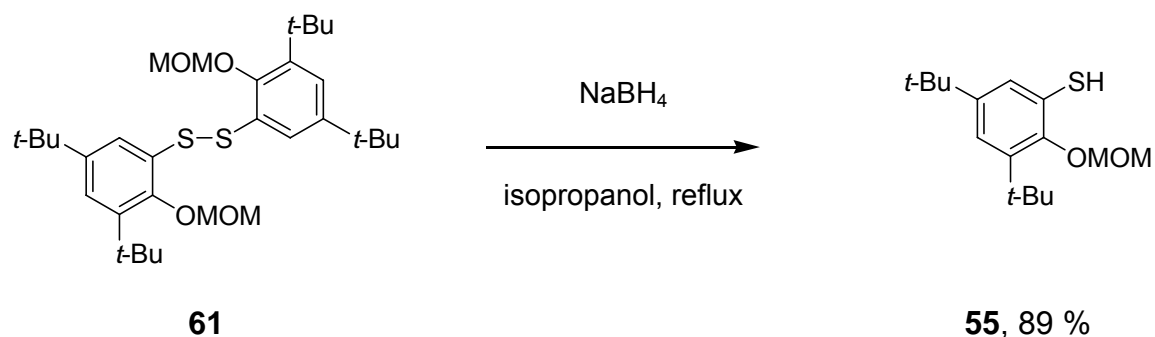
Scheme 5-9: Reduction of 1,1'-bis-[3,5-di-*tert*-butyl-2-(methoxymethoxy)phenyl]-disulfane 61 with sodium borohydride.[106]
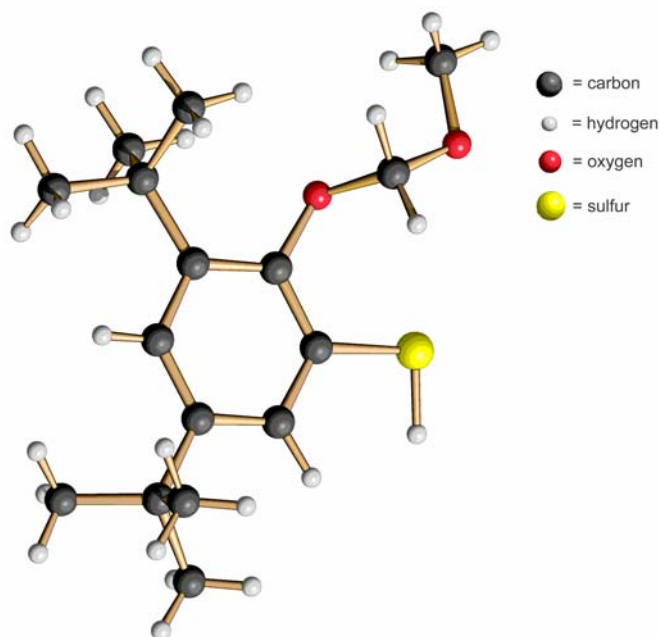
Figure 5-4: X-ray structure of 3,5-di-*tert*-butyl-2-(methoxymethoxy)benzenethiol 55.

5.1.2.4 Synthesis of *N*-protected *L*-serine-β-lactones

A *N*-protected *L*-serine-β-lactone is necessary as a second building block for the synthesis of *mod*-Cys according to the second strategy. The lactones **57** and **58** were synthesised as described by *Vederas* and co-workers, following a *Mitsunobu* cyclisation.[95] Boc and Cbz were chosen as protecting groups for the amine function since they are stable under the basic conditions of the ring opening.

Starting from the *N*-Boc-*L*-serine **59**, the *N*-Boc-*L*-serine-β-lactone **57** was formed by addition of **59** to the preformed adduct of triphenylphosphine and diethylazodicarboxylate at –50 °C. The lactone **57** was isolated, without racemisation in 55 % yield.
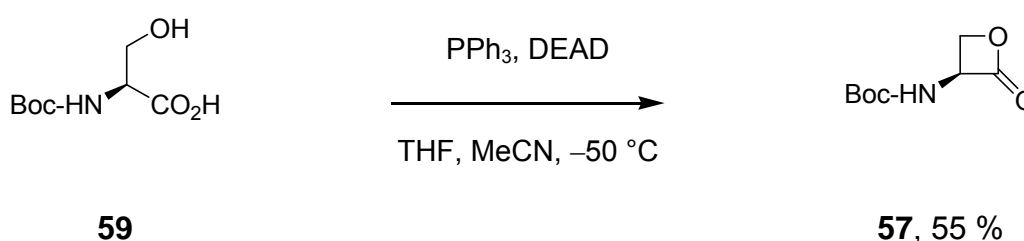
Scheme 5-10: Cyclisation of *N*-(*tert*-butyloxycarbonyl)-*L*-serine 59 by a *Mitsunobu* reaction.[95, 107]

Following the same protocol, lactone **58** was formed by cyclisation of *N*-Cbz-*L*-serine **60**. The addition of **60** to the preformed adduct of triphenylphosphine and di-*iso*-propylazodicarboxylate gave lactone **58** without racemisation in 49 % yield. The choice of the azodicarboxylate compound (DEAD versus DIAD) comes from the easiness of separation by column chromatography of the product from the by-product H_2-DIAD or H_2-DEAD.
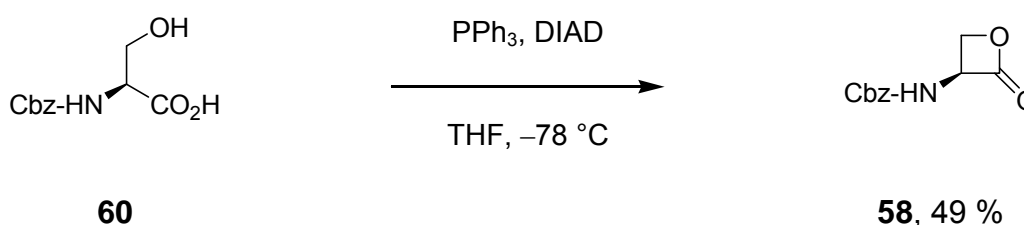
Scheme 5-11: Cyclisation of *N*-(benzyloxycarbonyl)-*L*-serine 60 by a *Mitsunobu* reaction.[95]

5.1.2.5 Opening of the lactones with 3,5-di-*tert*-butyl-2-(methoxymethoxy)-benzenethiol **55**

The lactones **57** and **58** were opened by the thiol **55** under basic conditions, as described by *Vederas* and co-workers.[95] Boc-*L-mod*-Cys(MOM)-OH **47** and Cbz-*L-mod*-Cys(MOM)-OH **53** were obtained in 85 % and 94 % yield respectively.

55   **57**   **47**, 85 %
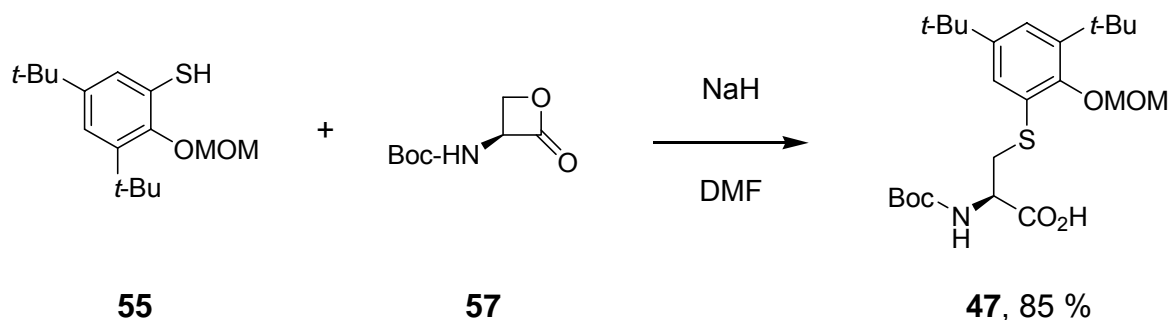
Scheme 5-12: Synthesis of Boc-*L-mod*-Cys(MOM)-OH 47 by ring opening of *N*-(*tert*-butyloxycarbonyl)-*L*-serine-β-lactone 57.[89, 95]

55   **58**   **53**, 94 %
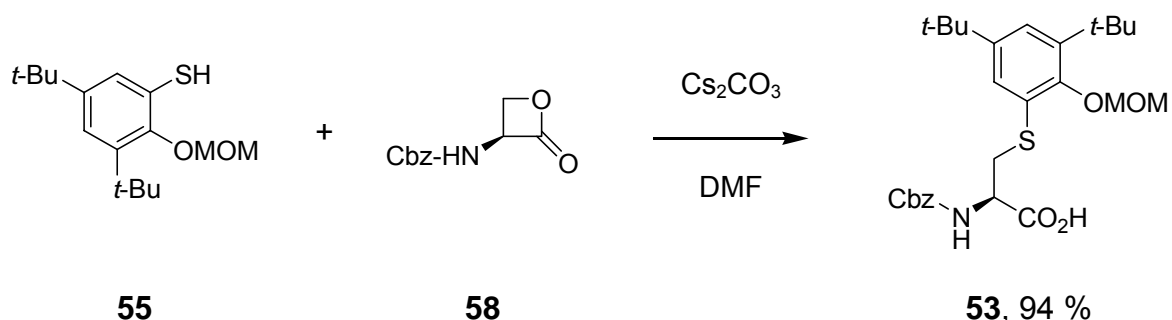
Scheme 5-13: Synthesis of Cbz-*L-mod*-Cys(MOM)-OH 53 by ring opening of *N*-(benzyloxycarbonyl)-*L*-serine-β-lactone 58.[95, 108]

5.1.2.6 Attempted deprotection of the Boc protecting group of Boc-*L-mod*-Cys(MOM)-OH **47**

The use of amino acids in peptide synthesis is easier when their amino function is protected by a Fmoc protecting group, thus allowing automation by quantitative evaluation of the coupling steps. The *mod*-Cys amino acid has so far been synthesised with Boc or Cbz protecting group on the amino function (products **47** and **53**). The replacement of Boc or Cbz by a Fmoc protecting group would thus be of interest.

When cleaving the Boc or Cbz protecting group, the MOM protecting group on the hydroxyl function must remain unchanged.

The Boc protecting goup is cleaved under anhydrous acidic conditions with TFA, while the MOM protecting group is cleaved under aqueous acidic conditions. Thus, by keeping anhydrous conditions, the Boc protecting group should be removed, while the MOM protecting group should not be cleaved.[109] Boc-*L-mod*-Cys(MOM)-OH **47** was treated with TFA in dry dichloromethane. Unfortunately, the expected product **76** was not obtained, but only the product **42** where both Boc and MOM protecting groups were cleaved.
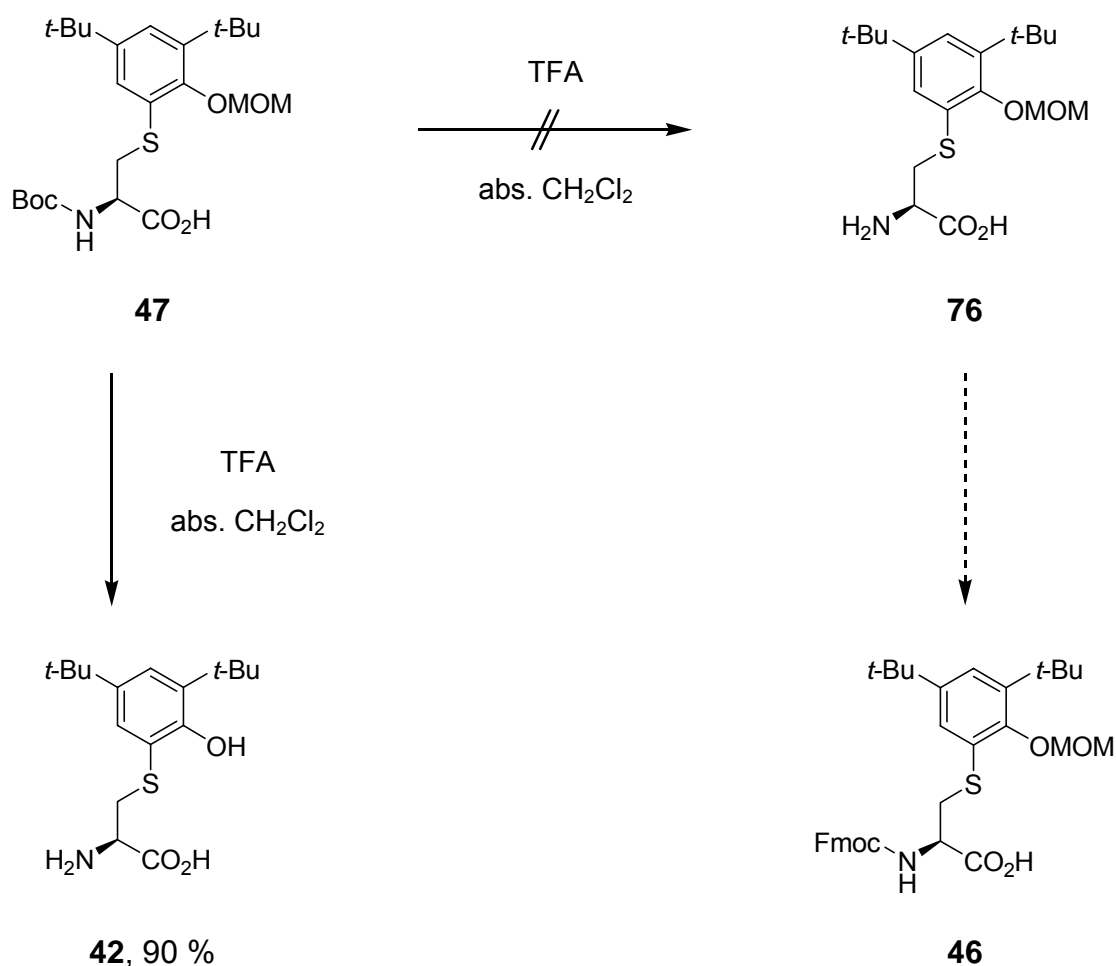
Scheme 5-14:   Treatment of Boc-*L-mod*-Cys(MOM)-OH 47 with TFA.[109]

Using various working-up conditions did not allow the isolation of the *N*-deprotected *O*-MOM-protected *mod*-Cys derivate **76**. When trying to prepare the *N*-Fmoc protected *mod*-Cys **46** in situ, the product which was obtained did not contain any MOM protecting group.

5.1.2.7 Attempted deprotection of the Cbz protecting group of

Cbz-*L-mod*-Cys(MOM)-OH **53**

The Cbz protecting group is usually cleaved under catalytic hydrogenolysis.[110, 111] Cbz-*L-mod*-Cys(MOM)-OH **53** was treated with hydrogen, using palladium on charcoal as a catalyst. However after 3 days, the starting material **53** was re-isolated.
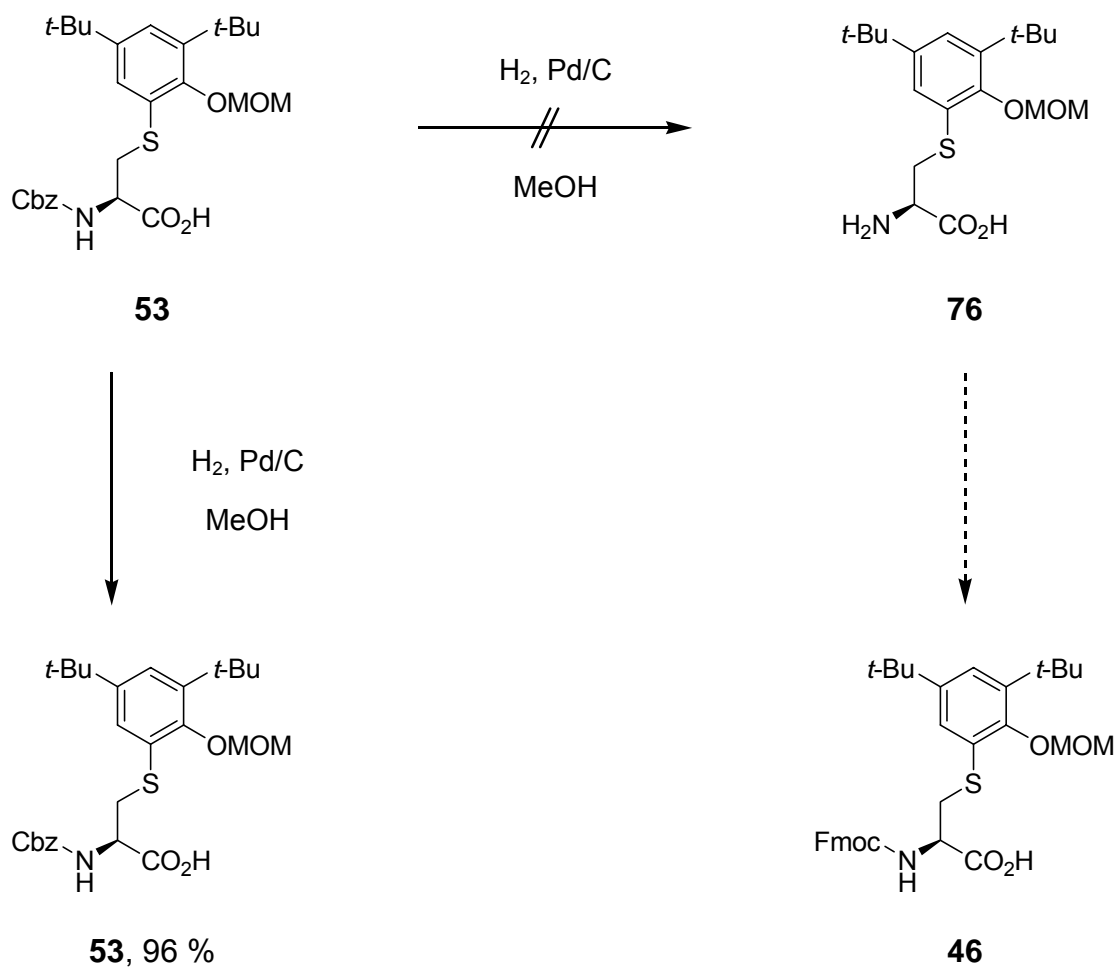
Scheme 5-15: Catalytic hydrogenolysis of Cbz-*L-mod*-Cys(MOM)-OH 53.[110, 111]

The reaction was carried out in various solvents (methanol, ethanol, dichloromethane or liquid ammonia)[110], under 1 bar and under 50 bar of dihydrogen. In all cases, the starting material **53** was re-isolated.

5.2 Synthesis of two solid-phase-bound *mod*-Cys-containing decapeptides, complexation with copper and use as catalysts in the aerobic oxidation of primary alcohols

5.2.1 Synthesis of the two copper complexes 84 and 85 of solid-phase-bound decapeptides containing the *mod*-Cys amino acid

Fmoc-*L*-*mod*-Cys(MOM)-OH **46** was not obtained. Nevertheless, Boc-*L*-*mod*-Cys(MOM)-OH **47** could be used in the synthesis of peptides.

To show that it was possible to incorporate *mod*-Cys into a peptide sequence, the decapeptides **78** and **79** were synthesised on amino TentaGel resin with a loading of 0.25 mmol/g (**Scheme 5-16**). Both peptides contained one *mod*-Cys amino acid, one tyrosine and two histidines, as it is the case at the active site of the enzyme GOase.

For the synthesis of the decapeptides, the functional groups on the side chains of tyrosine and histidine had to be temporally protected.

A specificity of this synthesis is the use of *N*-Fmoc-protected amino acids (His, Tyr, Phe, Gly, *D*-Pro) besides Boc-*L*-*mod*-Cys(MOM)-OH **47** which is a *N*-Boc-protected amino acid. The coupling steps were carried out following the normal procedure using PyBOP and NMM in DMF.[112] After each step, the *N*-protecting group was removed and the next coupling step was carried out under the same conditions. The Fmoc protecting groups were cleaved under basic conditions with 20 % piperidine[43] and the Boc protecting groups were cleaved under acidic conditions with 10 % TFA.[113] The syntheses were carried out in *Merrifield* flask, thus allowing the filtration of the reagents after each reaction step.

Fmoc-*L*-Phe-OH

Fmoc-Gly-OH

Fmoc-*D*-Pro-OH

Fmoc-*L*-His(Trt)-OH

Fmoc-*L*-Tyr(*t*-Bu)-OH

Boc-*L*-*mod*-Cys(MOM)-OH
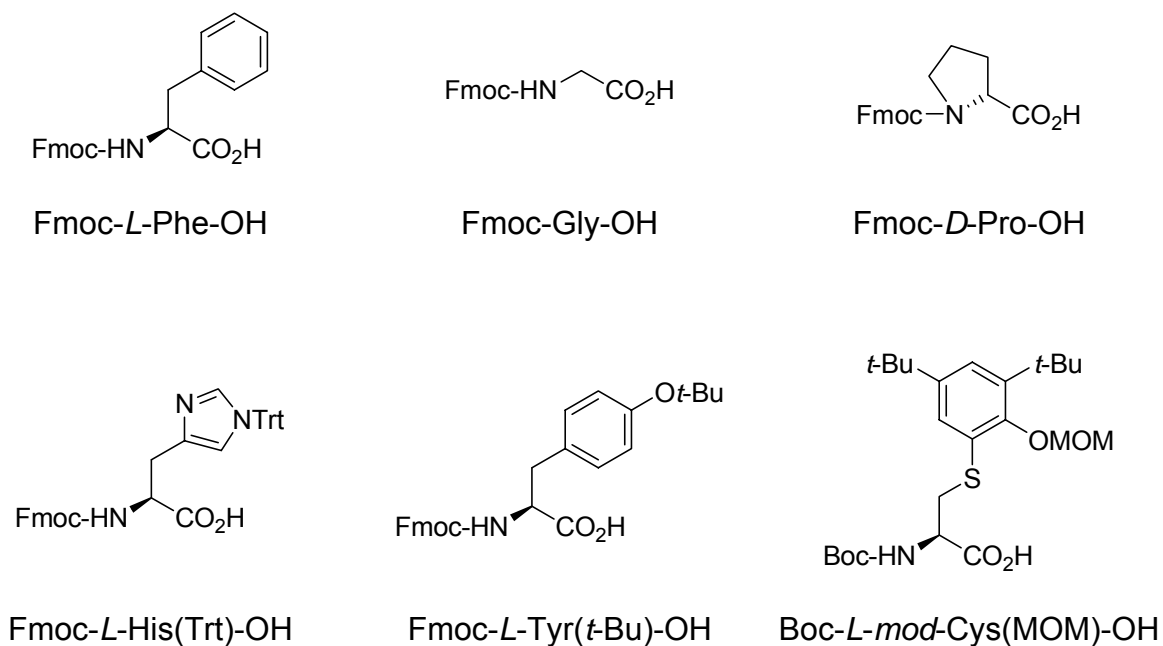
Figure 5-5: Amino acids used for the synthesis of the decapeptides 78 and 79.

77

1) swell in DMF

2) 2.50 eq AA, 2.50 eq PyBOP, 3 % NMM in DMF

3) test for free amino functions

4) 20 % piperidine in DMF or 10 % TFA in CH$_2$Cl$_2$

5) repetition of the steps 2) to 4) with Fmoc-*L*-Phe-OH, Fmoc-Gly-OH, Boc-*L*-*mod*-Cys(MOM)-OH **47**, Fmoc-*L*-His(Trt)-OH, Fmoc-*L*-Tyr(t-Bu)-OH or Fmoc-*D*-Pro-OH until completion of the 2 decapeptides

	X_1 = *mod*-Cys	X_1 = *mod*-Cys
	X_2 = Tyr(*t*-Bu)	X_2 = His(Trt)
	X_3 = His(Trt)	X_3 = His(Trt)
	X_4 = His(Trt)	X_4 = Tyr(*t*-Bu)
	78	**79**
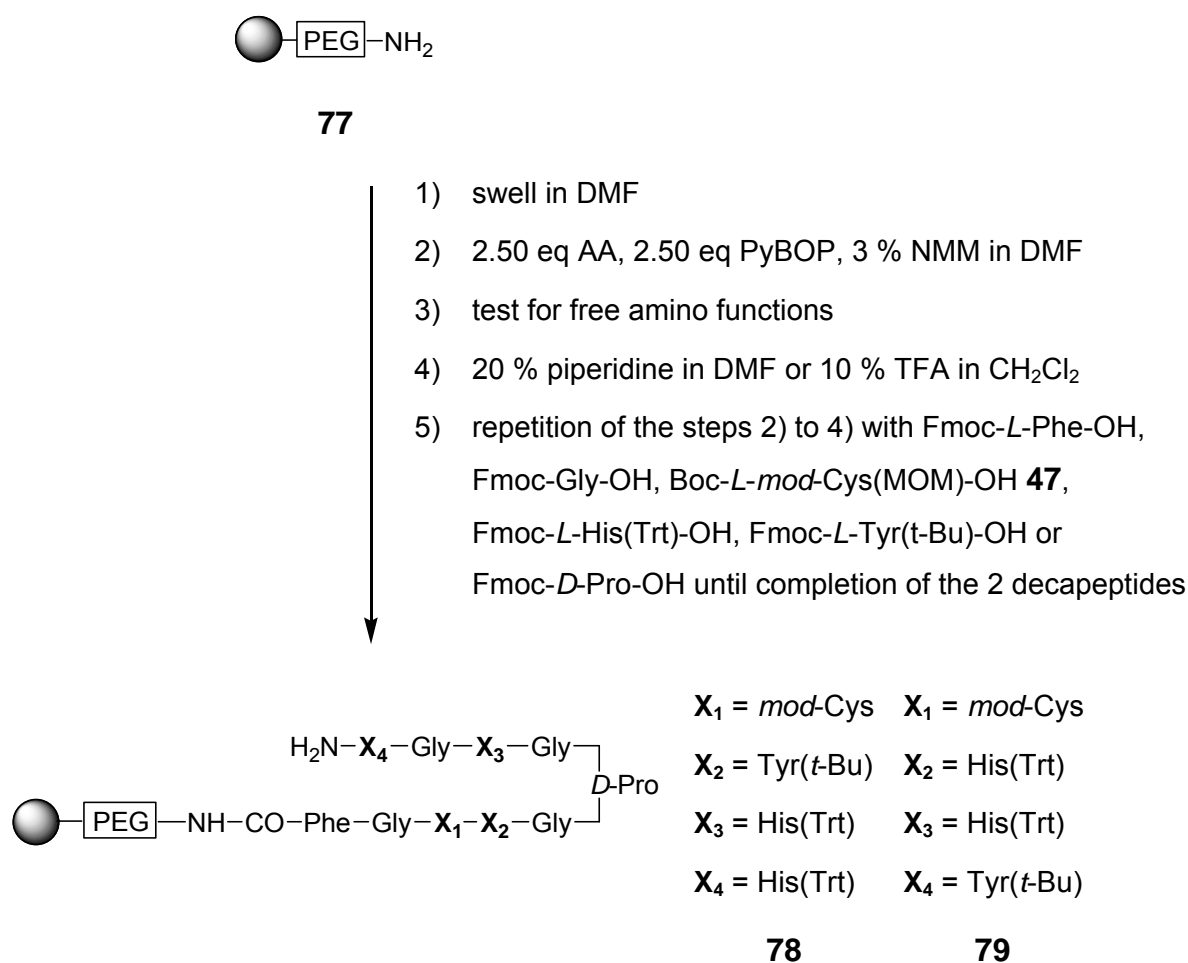
Scheme 5-16:   Synthesis of the two decapeptides 78 and 79 containing the unnatural *mod*-Cys amino acid on amino TentaGel resin (loading = 0.25 mmol/g).

The synthesis was followed by the colour qualitative test for free amino functions described by *De Clercq* and co-workers.[60] According to this test, a few beads were taken from the reaction flask and transferred into an acetonitrile solution of the active ester of disperse red **1**. The reaction mixture was heated and the beads were washed. The beads which contained free amino functions appeared red coloured while completely coupled beads (without free amino functions) remained colourless. When a red colour was observed (positive test), the coupling step was repeated (**Table 5-1**).
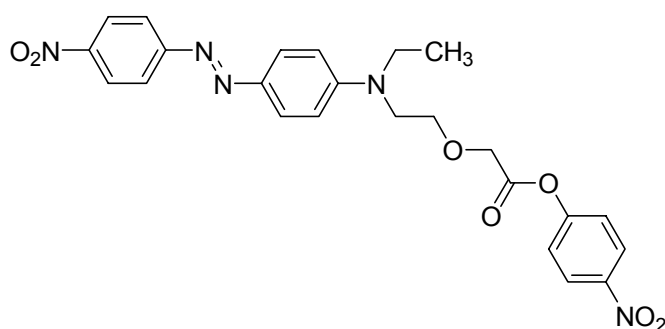
1[60]

Figure 5-6: NF31 used in the colour test for the detection of free amino functions.

Moreover, coupling steps including *N*-Fmoc protected amino acids could be quantified by UV-measurement of the dibenzofulvene-piperidine adduct formed during cleavage of the protecting group.[43] **Table 5-1** summarises the yields obtained in each coupling step.

The *N*-terminus of the decapeptides were acetylated by treatment with acetic anhydride and NMM in DMF (**Scheme 5-17**). The completion of the reaction was followed by the NF31 colour test.[60] When the colour test was carried out on the products **80** and **81**, it was negative. When the test was carried out on the starting material **78** and **79**, it was positive (**Figure 5-7**). From this test, it could be assumed that all the amino functions have been acetylated.

Table 5-1: **Yield for each coupling step in the synthesis of the decapeptides 78 and 79.**

peptide **78**		peptide **79**	
amino acid[a)]	yield [%]	amino acid[a)]	yield [%][c)]
Phe	100	Phe	100
Gly	94	Gly	94
mod-Cys	n.d.	*mod*-Cys	n.d.
Tyr[b)]	61	His[b)]	100
Gly	78	Gly	89
D-Pro	89	*D*-Pro	94
Gly[b)]	94	Gly[b)]	111
His	94	His	94
Gly	50	Gly	67
His	44	Tyr	61

[a)] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

[b)] The coupling step was repeated after observation of a positive colour test.

[c)] The values are relative to the first value which was fixed to 100 %.

Figure 5-7: Resin beads after treatment with NF31 colour test. Left: test carried out on the peptide 78 with free amino function. Right: test carried out on the acetylated peptide 80.

In the next step, the side chain protecting groups were cleaved by two successive treatments with TFA (**Scheme 5-17**). Triethylsilane was added to the cleavage

mixture to scavenge the cations formed during the acidic deblocking of the trityl and *tert*-butyl protecting groups.[113]

H₂N−**X₄**−Gly−**X₃**−Gly⌐
                            D-Pro
⬤−PEG−NH−CO−Phe−Gly−**X₁**−**X₂**−Gly⌐

X_1 = mod-Cys	X_1 = mod-Cys
X_2 = Tyr(*t*-Bu)	X_2 = His(Trt)
X_3 = His(Trt)	X_3 = His(Trt)
X_4 = His(Trt)	X_4 = Tyr(*t*-Bu)
78	**79**

1) Ac₂O, 3 % NMM in DMF

2) test for free amino functions

H₃C−C(=O)−N(H)−**X₄**−Gly−**X₃**−Gly⌐
                                     D-Pro
⬤−PEG−NH−CO−Phe−Gly−**X₁**−**X₂**−Gly⌐

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr(*t*-Bu)	X_2 = His(Trt)
X_3 = His(Trt)	X_3 = His(Trt)
X_4 = His(Trt)	X_4 = Tyr(*t*-Bu)
80	**81**

90 % TFA, 5 % H₂O, 5 % TES

H₃C−C(=O)−N(H)−**X₄**−Gly−**X₃**−Gly⌐
                                     D-Pro
⬤−PEG−NH−CO−Phe−Gly−**X₁**−**X₂**−Gly⌐

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr	X_2 = His
X_3 = His	X_3 = His
X_4 = His	X_4 = Tyr
82	**83**

1 eq Cu(OAc)₂, acetate buffer, MeOH

AcHN−**X₄**−Gly−**X₃**−Gly⌐
           Cu II   *D*-Pro
⬤−PEG−NH−CO−Phe−Gly−**X₁**−**X₂**−Gly⌐

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr	X_2 = His
X_3 = His	X_3 = His
X_4 = His	X_4 = Tyr
84	**85**

Scheme 5-17: Acetylation of the *N*-terminus of the decapeptides 78 and 79, deprotection of the side chains and complexation with copper(II).

Finally, the two peptide-ligands **82** and **83** were complexed with copper(II) by incubation with 1 equivalent of copper(II) acetate in a buffered methanol solution (**Scheme 5-17**).[90] After washing off with methanol, the resin beads showed a green-blue colour (**Figure** 5-8). This colour is an indication that the complexation with copper was successful. By comparison, the on-bead peptide-ligands **82** and **83** appeared to be pale yellow coloured. Moreover, when acetylated amino TentaGel resin **86** was incubated in a copper(II) acetate buffered methanol solution and washed with methanol, the beads remained with a pale yellow colour (**Scheme 5-18**). This indicated that the copper ion was bound neither to the polyethylene glycol spacer of the resin, nor to the resin itself.
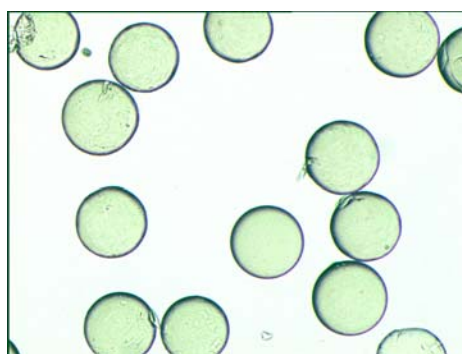
Figure 5-8: Resin beads loaded with copper complex 84.

86
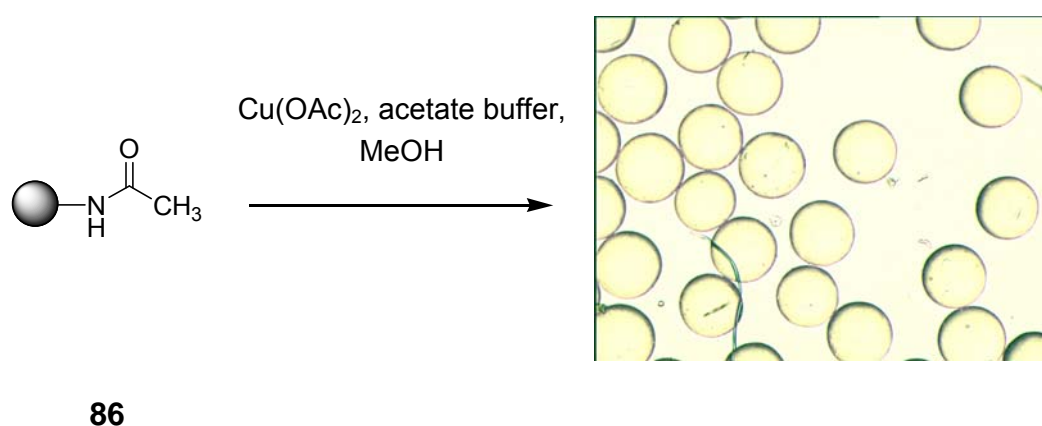
Scheme 5-18: Incubation of acetylated amino TentaGel 86 with copper and observation of the resin beads under the microscope.[90]

5.2.2 Catalysis with the *mod*-Cys containing peptide–copper(II) complexes 84 and 85.

The peptide–copper(II) complexes **84** and **85** were tested in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol **39**. In the first catalytic reaction, the peptide–copper(II) complexes were used without pre-activation to the radical–copper(II) state. Both peptide–copper(II) complexes **84** and **85** were compared. The catalysis was performed at room temperature with 0.50 mol% of copper complex and 7.00 mM of substrate in acetonitrile / water (1:1). The flask was filled with dioxygen and closed. 5.00 mol% of potassium hydroxide was added to the reaction mixture in order to estimate its influence on the catalysis. The yield in 3-methoxybenzaldehyde **40** was measured by following the absorbance of the solution at λ = 310 nm.[64, 98] The results are summarised in **Table 5-2**.
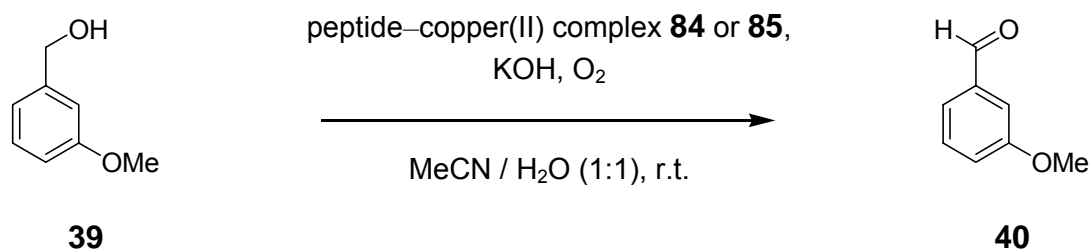
Scheme 5-19: Catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39 with peptide–copper(II) complexes 84 and 85.[64, 98]

Table 5-2: **3-Methoxybenzaldehyde formed after 24 hours by catalytic aerobic oxidation of 3-methoxybenzyl alcohol with peptide–copper(II) complexes 84 and 85.**

entry	peptide–copper(II) complex		KOH	3-methoxybenzaldehyde after 24 h	yield	TON
		[mol%]	[mol%]	[μmol]	[%]	
1	-	-	-	0.14	0.39	<1
2	-	-	5.00	0.36	1.01	2.0
3	**84**	0.50	-	0.15	0.41	<1
4	**85**	0.50	-	0.13	0.36	<1
5	**84**	0.50	5.00	0.30	0.83	1.7
6	**85**	0.50	5.00	0.38	1.06	2.1

Without addition of potassium hydroxide (entry 3 and 4), no catalytic activity was observed. The activity that was observed with the copper complexes and potassium hydroxide (entry 5 and 6) is due only to the base since the same activity was observed when no copper complex was used (entry 2).

Moreover, the catalysis was performed in neat 3-methoxybenzyl alcohol **39**. Here the influence of the bases triethylamine and potassium hydroxide were compared. The catalysis was performed with 0.10 mol% of copper complex and 20 mol% of base at room temperature in a flask filled with dioxygen. The yield in 3-methoxybenzaldehyde **40** was measured by GC. The activity of the peptide–copper(II) complexes were also compared to the activity of the acetylated amino TentaGel **86**. The results are summarised in **Table 5-3**.
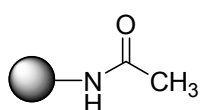
86

Figure 5-9: Acetylated aminoTentaGel 86.
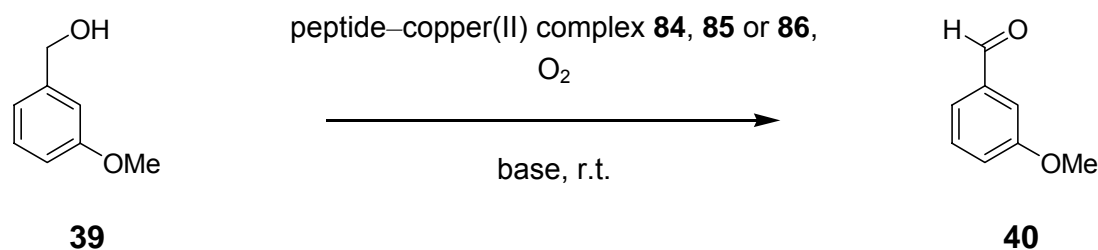
Scheme 5-20: Catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39 with peptide–copper(II) complexes 84 and 85.

Table 5-3: Influence of the base on the catalytic aerobic oxidation of neat 3-methoxybenzyl alcohol 39 with peptide–copper(II) complexes 84 and 85.

entry	catalyst	[mol%]	base	[mol%]	3-methoxybenzaldehyde[a] after 24 h [μmol]	yield [%]	TON
1	**84**	0.10	-	-	-	-	-
2	**85**	0.10	-	-	-	-	-
3	**84**	0.10	NEt$_3$	20.0	-	-	-
4	**85**	0.10	NEt$_3$	20.0	-	-	-
5	-	-	KOH	20.0	0.10	0.06	<1
6	**86**[b]	0.10	KOH	20.0	1.93	1	10
7	**84**	0.10	KOH	20.0	6.28	4	40
8	**85**	0.10	KOH	20.0	4.51	3	30

[a] 3-Methoxybenzoic acid was not formed.

[b] 0.10 mol% of copper(II) acetate were added to the reaction mixture.

When only 0.10 mol% of a catalyst in pure substrate was used, a turnover number of 40 was obtained with the peptide–copper(II) complex **84** (entry 7). The addition of potassium hydroxide was necessary for the catalytic activity while the use of triethylamine as a base deactivated the catalyst. Without any base, the formation of 3-methoxybenzaldehyde **40** was not observed (entry 1 and 2). With 20 mol% of

triethylamine, no conversion was observed for the both complexes (entry 3 and 4). Potassium hydroxide alone showed a very low activity: less than 1 turnover number was observed after 24 hours (entry 5). Acetylated amino TentaGel, together with copper(II) acetate, showed a lower activity than the peptide–copper(II) complexes **84** and **85**. A turnover number of 10 was observed for the acetylated amino TentaGel (entry 6). Turnover numbers of 40 and 30 were observed for the complexes **84** and **85** respectively (entry 7 and 8).

5.3 Synthesis of *N*-(9-fluorenylmethoxycarbonyl)-2,2,6,6-tetramethyl piperidine-1-oxyl-4-amino-4-carboxylic acid (Fmoc-TOAC-OH) 43

The TOAC amino acid **41** is an alternative for the *mod*-Cys amino acid as a mimic of the Tyr–Cys cross-link found at the active site of GOase. Indeed, TOAC is an amino acid derived from the free radical TEMPO. TEMPO, together with copper(II), catalyses the aerobic oxidation of alcohols.[10] The incorporation of TOAC in the peptidic chain, which contains also the amino acids histidine and tyrosine, would lead to a structural mimic of GOase. *Toniolo* and co-workers have already shown the possibility of incorporating TOAC into a peptidic chain.[114] A temporary protection of the nitroxyl radical is not necessary.
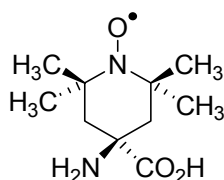
41

The *N*-Fmoc-protected TOAC amino acid **43** was synthesised in four steps starting from the commercially available 2,2,6,6-tetramethyl-4-oxo-piperidine **87** and according to the procedure described by *Dulog* and co-workers.[93]

In the first step, the secondary amine **87** was oxidised to its nitroxyl radical **88** with hydrogen peroxide and sodium tungstate. The stable nitroxyl radical was obtained in 93 % yield and recrystallised from *n*-hexane. The orange needles obtained were suitable for X-ray analysis (**Figure 5-9**).
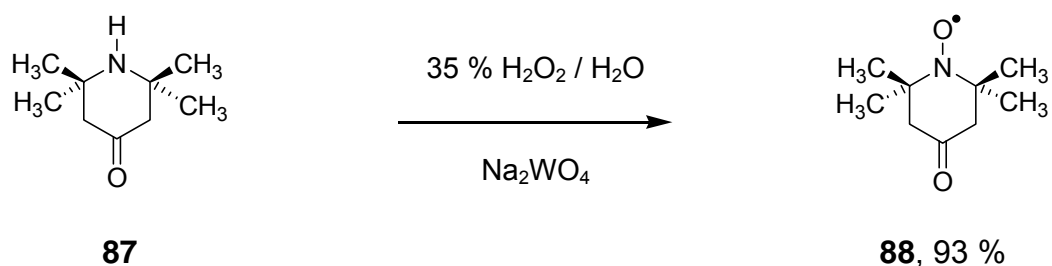
Scheme 5-21: **Formation of the nitroxyl radical 88 from 2,2,6,6-tetramethyl-4-oxo-piperidine 87.**[93]
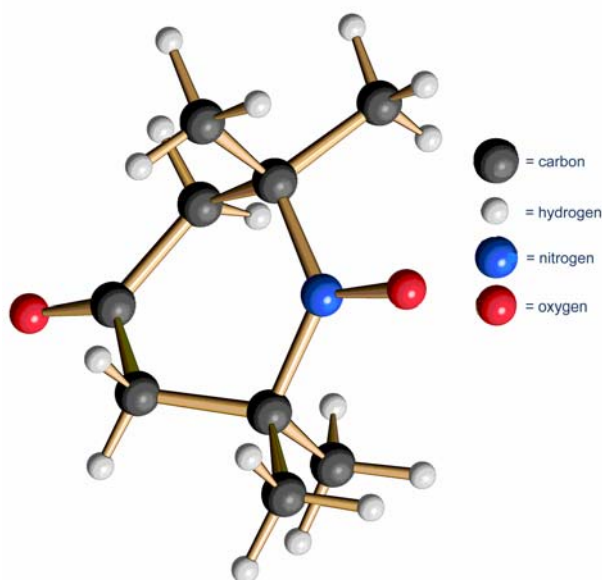
Figure 5-10: **X-ray structure of 2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl 88.**

The amino acid **41** was prepared from the ketone **88** applying the Bucherer–Bergs synthesis.[115] Reaction of the ketone **88** with sodium cyanide and barium hydroxide in a mixture of ethanol and water at 60 °C and overnight gave the hydantoin **89** in 83 % yield (**Scheme 5-22**). Alkaline hydrolysis of the hydantoin with barium hydroxide resulted in the TOAC amino acid **41** with 88 % yield (**Scheme 5-23**).
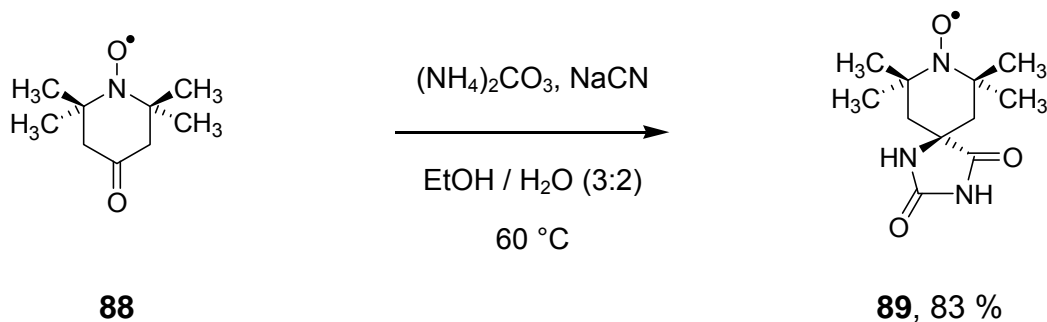
Scheme 5-22: Bucherer–Bergs synthesis with 2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl **88**.[93, 115]
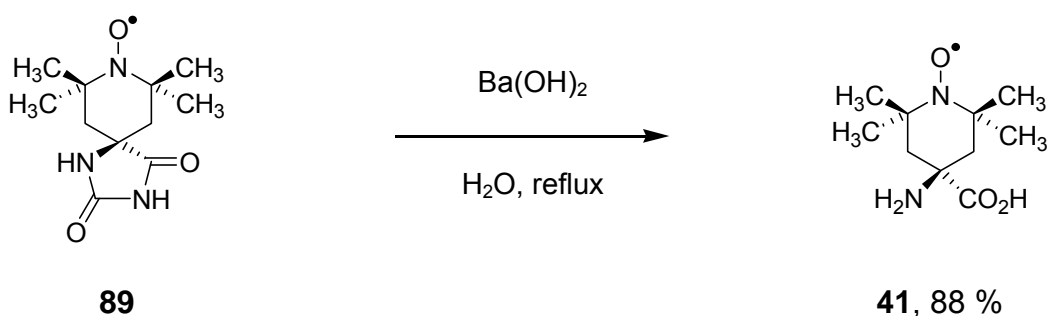
Scheme 5-23: Synthesis of 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) **41** from the hydantoin **89**.[93, 115]

At the final stage of the synthesis, TOAC **41** was *N*-protected with Fmoc.[116] This reaction was carried out under basic conditions, using Fmoc-OSu in a mixture of water and 1,4-dioxane. After stirring the solution at room temperature, followed by an acidic work up, a mixture of Fmoc-TOAC **43** and its hydroxylamine derivative **90** was obtained. The two of them could be separated by column chromatography and Fmoc-TOAC **43** was recrystallised from a mixture of *n*-hexane and ethyl acetate to give little yellow needles. A crystal structure of **43** was obtained (**Figure 5-11**).
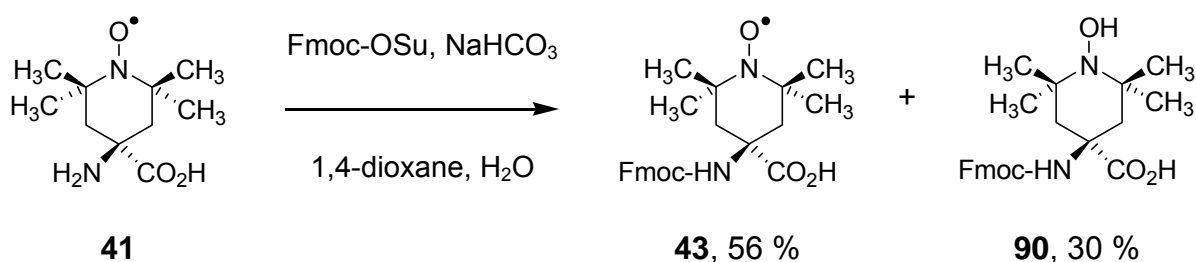
Scheme 5-24: *N*-protection of TOAC **41** with Fmoc.[116]
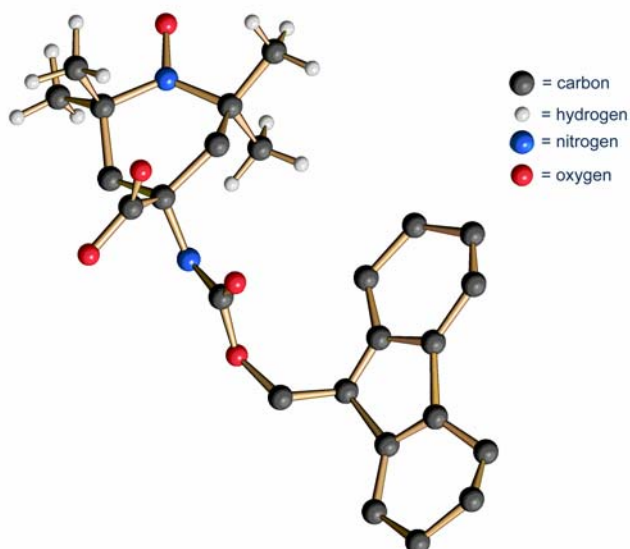
Figure 5-11: X-ray structure of Fmoc-TOAC 43.

5.4   Synthesis of the peptide library

In order to check whether TOAC could be quantitatively coupled to a peptide chain, the tripeptide **92** was synthesised on *Wang* resin, cleaved from the resin and analysed by LC–MS.

The *Wang* resin was loaded with phenylalanine. The first coupling on the hydroxy function of a *Wang* resin was carried out efficiently using di-*iso*-propylcarbodiimide and HOBt as a coupling agent.[48] The coupling of glycine and TOAC took place by the standard PyBOP procedure.[112] The three coupling steps were monitored with the NF31 colour test,[60] as well as by UV measurement of the dibenzofulvene–piperidine adduct after cleavage of the Fmoc protecting groups.[43]

The colour test for free amino functions was negative after each coupling step, thus indicating quantitative couplings for the three amino acids. The UV measurements of the dibenzofulvene–piperidine adduct confirmed that the first two amino acids (Phe and Gly) had been coupled almost quantitatively (91 % and 100 % yield for Phe and Gly respectively). However at the third coupling step, the UV value did not match the colour test. Indeed, the UV value suggested a yield of 68 %.
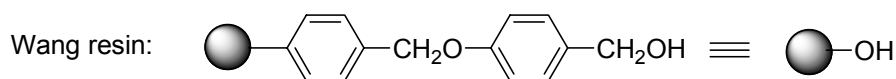
Wang resin: (structure) ≡ (●)—OH

91

1) swell in DMF

2) 5.00 eq Fmoc-*L*-Phe-OH, 5.00 eq DIC,
   5.00 eq HOBt, 0.10 eq DMAP

3) test for free amino functions

4) 20 % piperidine in DMF

(●)—OH ——————————————————→
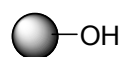
5) 2.50 eq   Fmoc-Gly-OH,   2.50 eq
   PyBOP, 3 % NMM in DMF

6) repetition of the steps 3) and 4), then
   5) with Fmoc-TOAC-OH and finally 3)
   and 4)
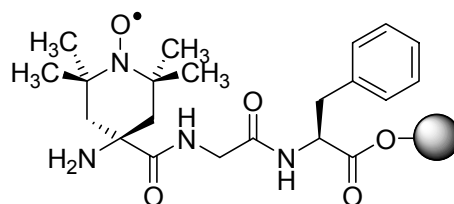
91   **92**

Scheme 5-25:   Synthesis of the tripeptide 92 containing the TOAC amino acid on *Wang* resin.

The tripeptide was cleaved from the *Wang* resin with two successive treatments with a mixture of TFA, H_2O and TIS.[26] The tripeptide was precipitated by addition of diethylether to the filtrate.

90 % TFA, 5 % H_2O,
5 % TIS
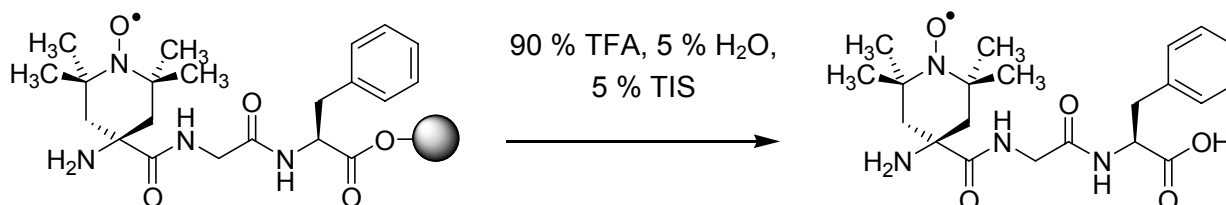
92   **93**

Scheme 5-26:   Cleavage of the tripeptide 92 from the *Wang* resin.

After filtration and lyophilisation, the product was analysed by LC–MS. Four peaks were observed on the chromatogram. Three of them correspond to the tripeptide

(m/z 419, m/z 420 and m/z 421) containing either the nitroxyl radical (**93**), or the hydroxyl amine function (**94**). Another product appeared on the chromatogram with a mass of 272, corresponding to the dipeptide H_2N-TOAC-Gly-CO_2H (**95**). However the dipeptide H_2N-Gly-Phe-CO_2H was not observed. These results indicated that the TOAC amino acid has been coupled quantitatively and that the results of the UV measurements are not reliable for the coupling of the TOAC amino acid.

93	**94**	**95**
419.23 g·mol^{-1}	420.50 g·mol^{-1}	272.32 g·mol^{-1}
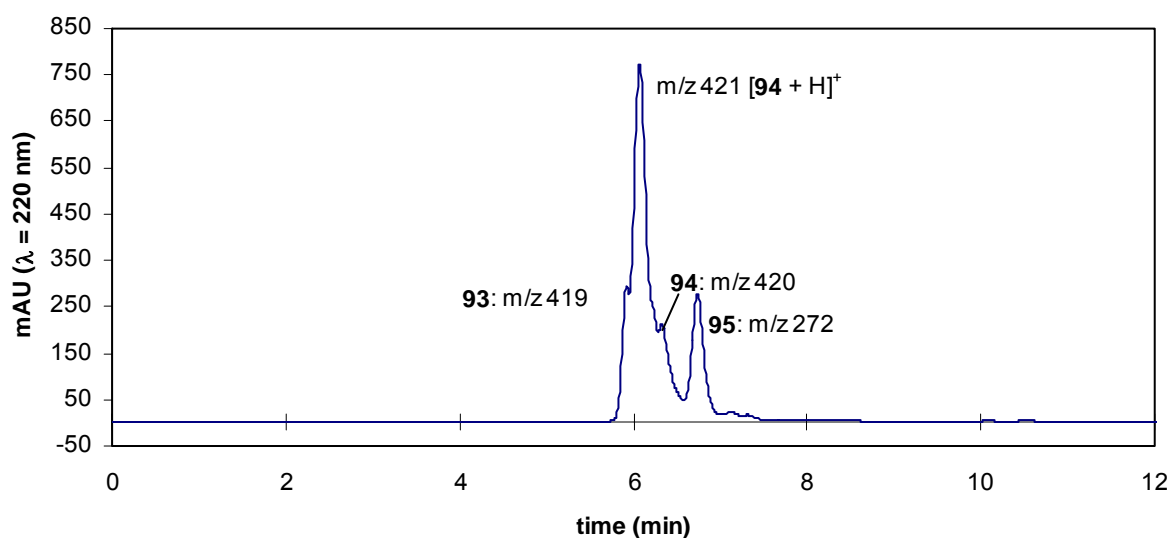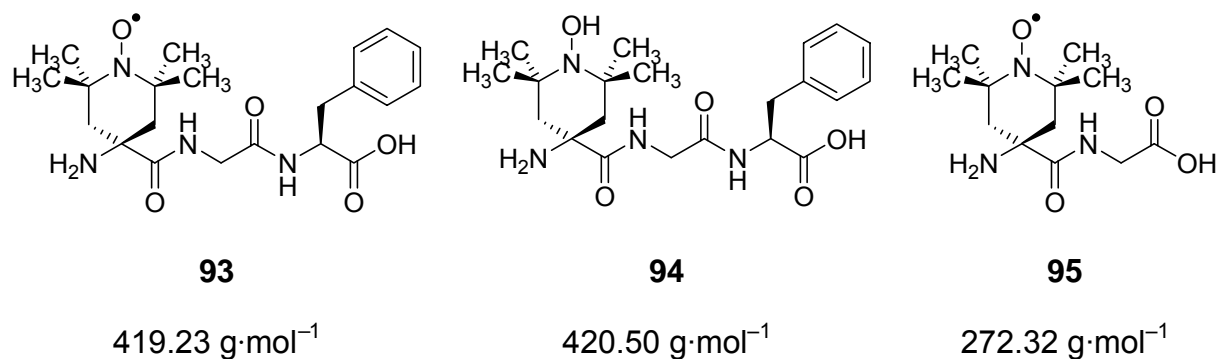
Figure 5-12: **Baseline corrected LC–MS diagram of the crude product obtained from cleavage of the tripeptide 92 (column: RP-18, mobile phase: MeCN : H_2O : AcOH = 90 : 10 : 0.5).**

According to the results of the synthesis of the tripeptide **93**, the library of decapeptides could be synthesised.

The 81-member peptide library **99** was synthesised on amino TentaGel resin with a loading of 0.26 mmol/g. The library was synthesised in a combinatorial fashion, following the split-mix strategy.[31] The *IRORI* radiofrequence tagging system was used to allow rapid deconvolution of the members of the library.[40]

81 *IRORI* MikroKans were filled with 30 mg each of amino TentaGel resin. In addition to the resin, an AccuTag was added and the Kan was closed. The Tags were encoded, i.e. to each Kan was asigned a code corresponding to one member of the library. During the synthesis and for the screening, it was always possible to identify the decapeptide present in a Kan.

The peptides were synthesised according to the Fmoc/PyBOP strategy.[43] Therefore couplings were performed with 3 % NMM in DMF, 2.5 equivalents of PyBOP as a coupling agent and 2.5 equivalents of amino acid (**Scheme 5-27**). Each step of the reaction was followed by colour test (NF31).[60] The coupling steps were repeated until a negative colour test (no free amino functions) was obtained. The coupling of the amino acids which were situated directly after TOAC, as well as the last amino acids of the sequences, had to be carried out by repeating the procedure three times. Each coupling step showed a negative colour test, indicating quantitative coupling yields.
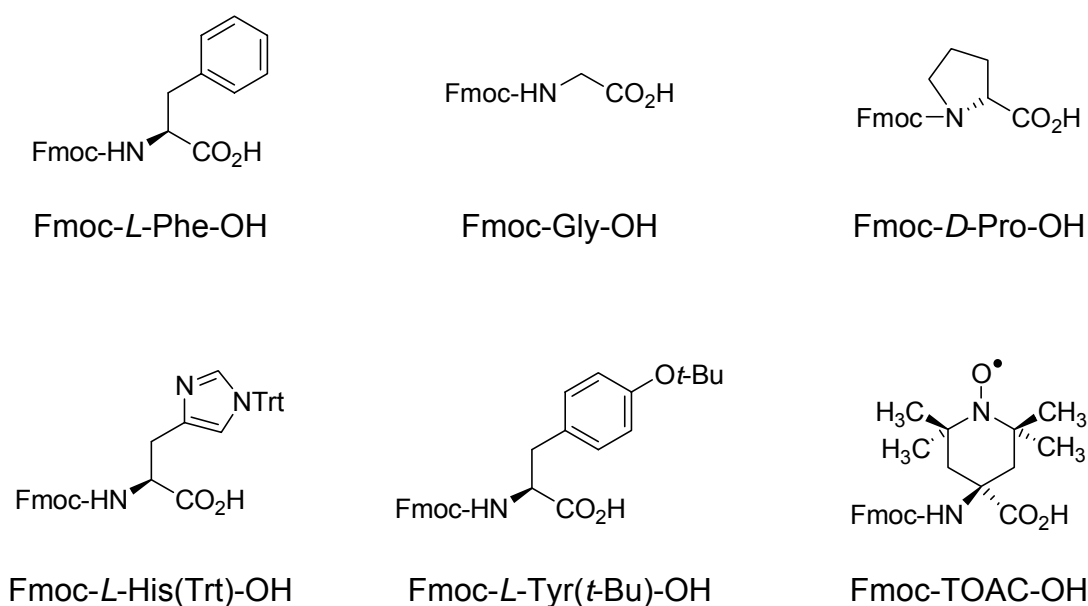
Figure 5-13: **Amino acids used for the synthesis of the 81-member library of decapeptides 96.**
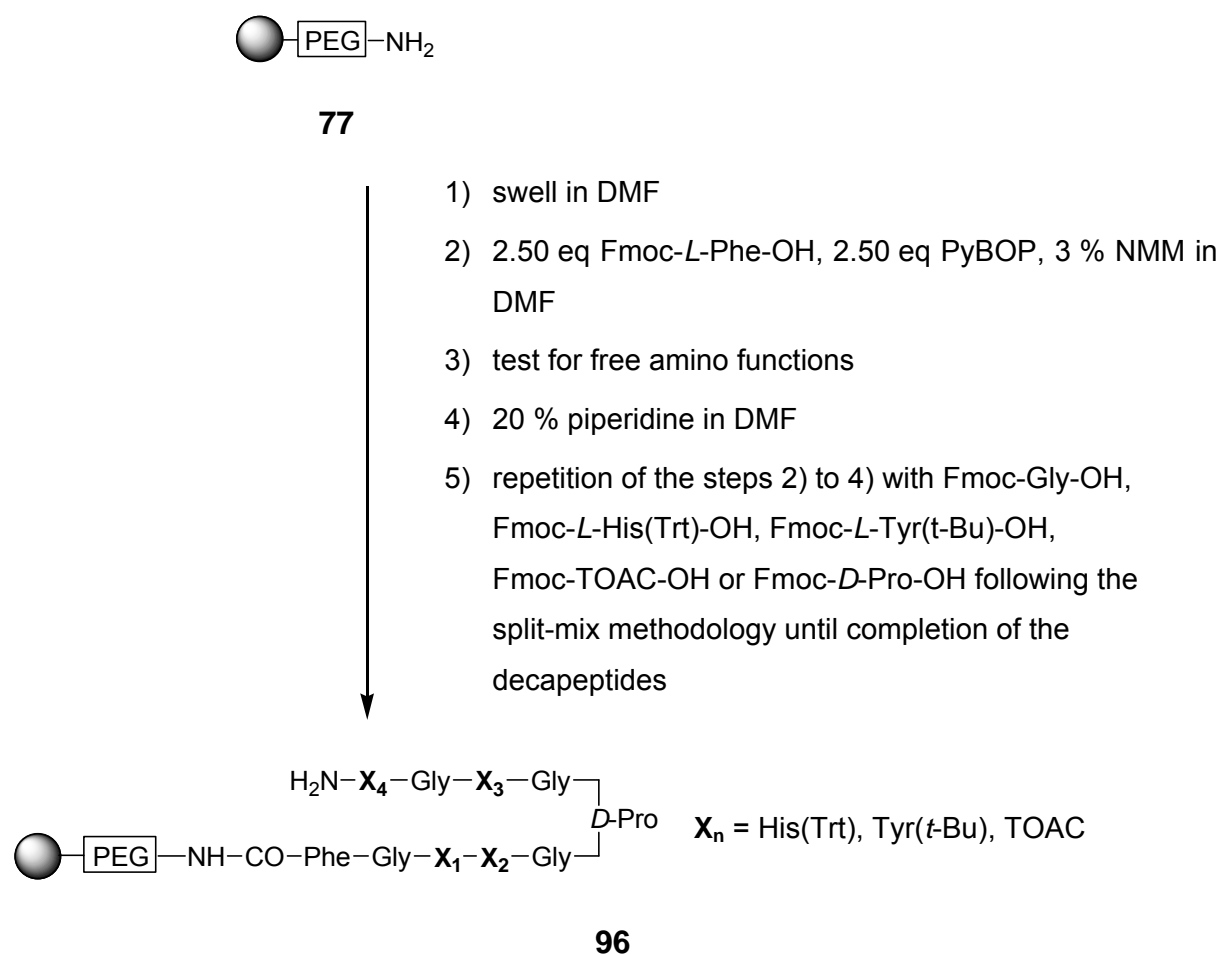
Scheme 5-27: Split-mix synthesis of the 81-member library of decapeptides 96 containing the unnatural amino acid TOAC on amino TentaGel resin.

The yields of the coupling steps were quantitatively defined by UV absorbance measurement of the dibenzofulvene–piperidine adduct after Fmoc cleavage with piperidine. The yields are summarised in **Table 5-4**. All the yields were above 85 %, except for the coupling of TOAC, which showed coupling yields between 50 and 80 %. However, according to the results obtained from the synthesis and the analysis of the tripeptide **93**, we can conclude that TOAC was coupled quantitatively, even if the UV measurements gave some lower values.

Table 5-4: Synthesis of the 81-member peptide library. Yield for each coupling step.

coupling	amino acid[a)]	yield [%]
1	Phe	100
2	Gly	97
3a	His	101
3b	Tyr	100
3c	TOAC	78
4a	His	103
4b	Tyr	101
4c	TOAC	53
5	Gly	108
6	*D*-Pro	99
7	Gly	103
8a	His	91
8b	Tyr	87
8c	TOAC	46
9	Gly	88
10a	His	88
10b	Tyr	97
10c	TOAC	57

[a)] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

The *N*-termini of the decapeptides were acetylated (**Scheme 5-28**). The completion of the reaction was checked by colour test (NF31).[60] Resin beads from **97** gave a negative colour test, while resin beads from **96** gave a positive colour test.
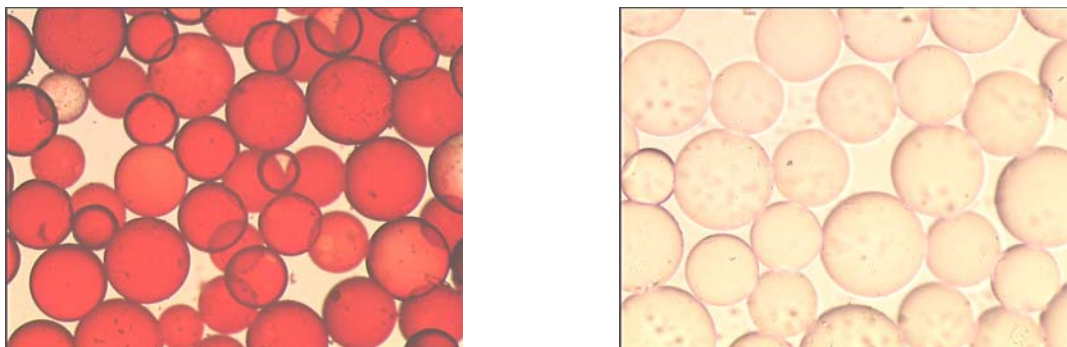
Figure 5-14: **Resin beads after treatment with NF31 colour test. Left: test carried out on the sample beads from library 96. Right: test carried out on the sample beads from the acetylated library 97.**

The side chain protecting groups (trityl from His and *tert*-butyl from Tyr) were cleaved with a mixture of TFA, water and TES.[113] TES was used to scavenge the cations formed during the cleavage. Following some remarks from *Toniolo*, nitroxyl radicals are converted to hydroxyl amine by treatment with TFA.[114] However, free radicals can be regenerated by treatment with aqueous ammonia. As a consequence, the peptides were treated with a 10 % aqueous ammonia solution in order to regenerate the nitroxyl radicals. After washing and drying, the peptide-ligands **99** were ready for catalysis.

96

1) Ac$_2$O, 3 % NMM in DMF

2) test for free amino functions

97

90 % TFA, 5 % H$_2$O, 5 % TES

98

10 % aq. NH$_3$
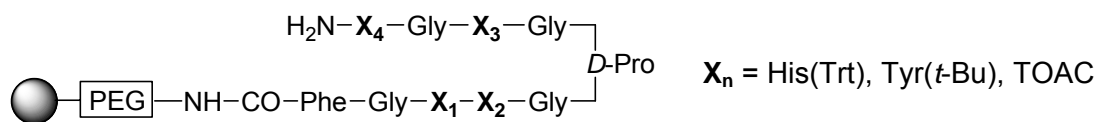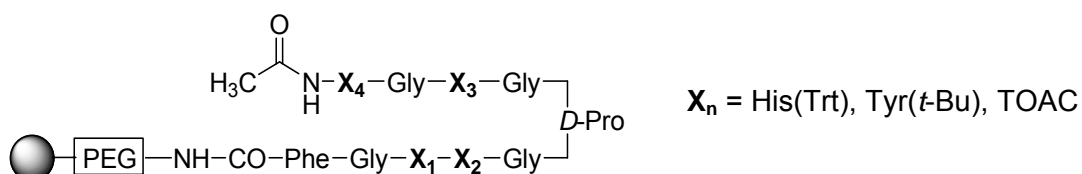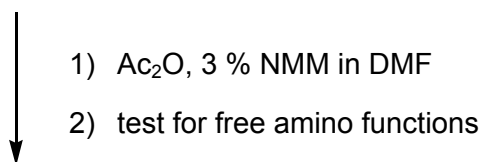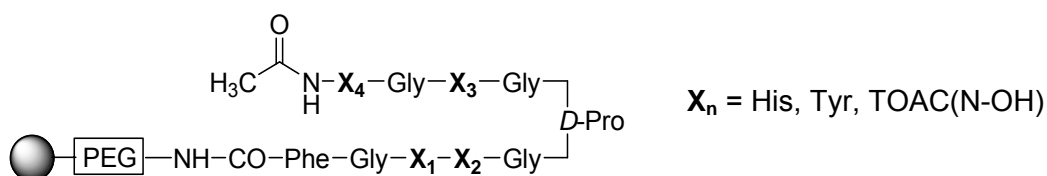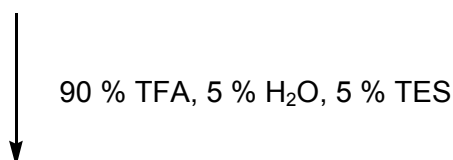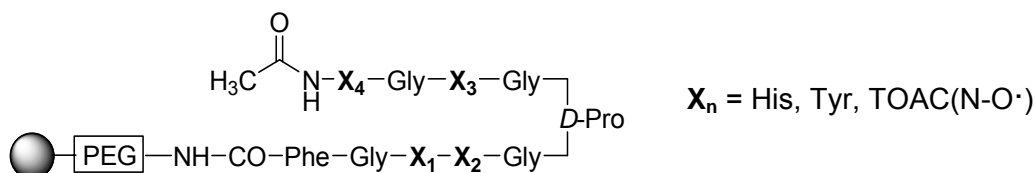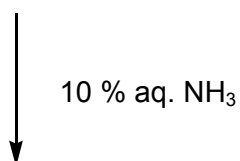
99

Scheme 5-28: Acetylation of the *N*-termini, cleavage of the side chain protecting groups and regeneration of the nitroxyl radical of the library of peptide-ligand 96.

The peptide-ligands were incubated with copper(II) acetate (**Scheme 5-29**).[90]

99

1 eq Cu(OAc)$_2$, acetate buffer, MeOH

100
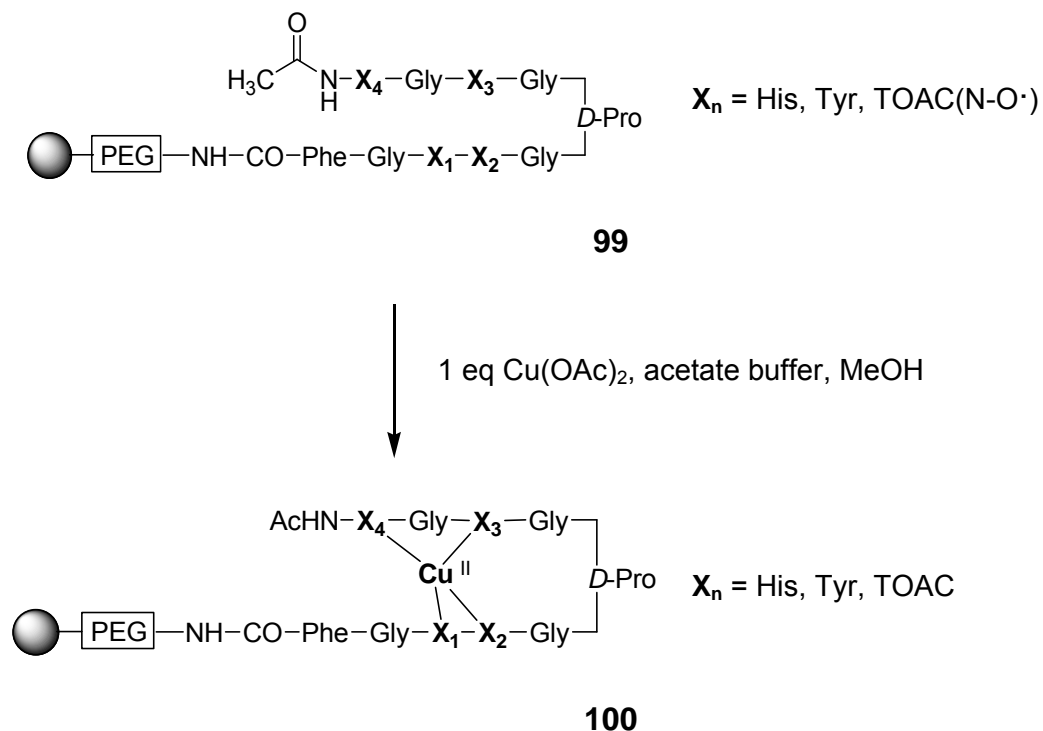
Scheme 5-29: Incubation of the library of peptide-ligand 99 with copper(II) acetate.

Upon complexation with copper(II) acetate, the resin beads carrying the different peptide-ligands appeared to be differently coloured. Moreover, incubation of an acetylated amino TentaGel resin with copper(II) acetate did not result in any change of the beads colour (5.2.1, **Scheme 5-18**). These colours showed that, (i) there is a complexation of the copper ion with the on-bead peptide-ligands, and (ii) the complexation takes place at the functional amino acids (Tyr, His and TOAC). Indeed a general trend in the colour of the beads could be observed (**Figure 5-15**): the on-bead peptide-ligands including some histidine (His) in their structure tend to form blue complexes (a), the one including some tyrosine (Tyr) gave rise to green complexes (b) and the one including some TOAC resulted in orange complexes (c).
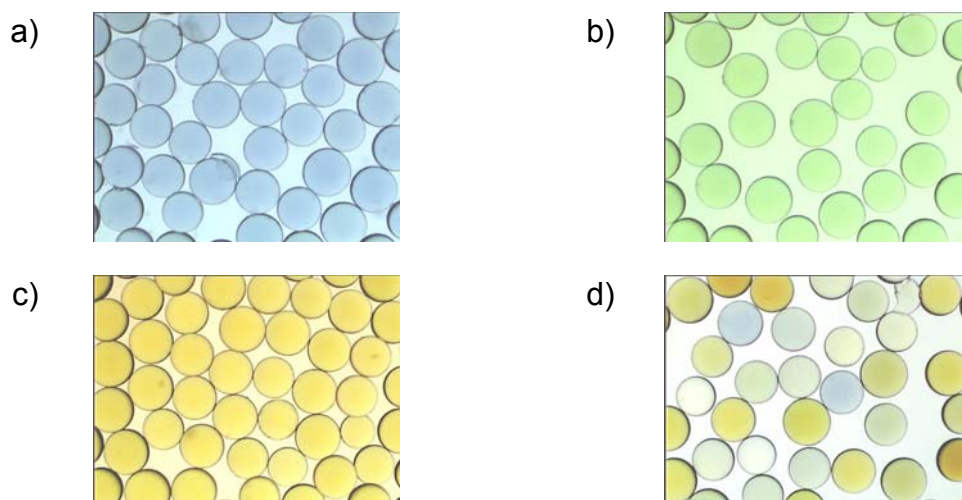
Figure 5-15: Resin beads of some members of the library 99 after incubation with copper(II) acetate. Peptide sequences X_1, X_2, X_3, X_4 = His, His, His, His (a), His, His, His, Tyr (b), TOAC, Tyr, Tyr, His (c). d) Mixture of the resin beads from the 81 members of the library.

5.5 Screening of the library for catalytic activity in the aerobic oxidation of primary alcohols

The library of peptide–ligands **99** was screened for activity using 3-methoxybenzyl alcohol **39** as the test substrate. This substrate was chosen because its oxidation product, 3-methoxybenzaldehyde **40**, absorbs in the UV at 310 nm while the alcohol does not absorb at this wavelength, as already described by *Branchaud* and *Kosman*.[64, 98] As a consequence, it was possible with this test substrate to carry out a rapid screening of the on-bead peptide–ligands for catalytic aerobic oxidation by simply following the UV absorbance of the reaction mixtures.

The 81 members of the library **99** were screened in parallel after decoding of the sequence with the *IRORI* tagging station. The on-bead peptide-ligands were taken out of the Kans to carry out the catalysis.

For the first screening, the oxidation was carried out in simple experimental conditions: no base was added and the reaction flask was simply opened to the air to allow dioxygen to react with the copper complexes. The oxidation occurred at room temperature in a mixture of acetonitrile and water (1:1).

The complexes were formed by the addition of the peptide-ligands **99** to a solution of copper(II) acetate in acetonitrile / water (1:1), and the substrate was added to this reaction mixture. No change of the absorbance at λ = 310 nm was observed after 24

hours when only 1 equivalent of copper versus peptide-ligand was used. Two more equivalents of copper were necessary in order to observe a formation of 3-methoxybenzaldehyde.

Therefore the oxidation was carried out with 1.00 mol% of peptide–ligand and 3.00 mol% of copper(II) acetate relative to the substrate (**Scheme 5-3**).
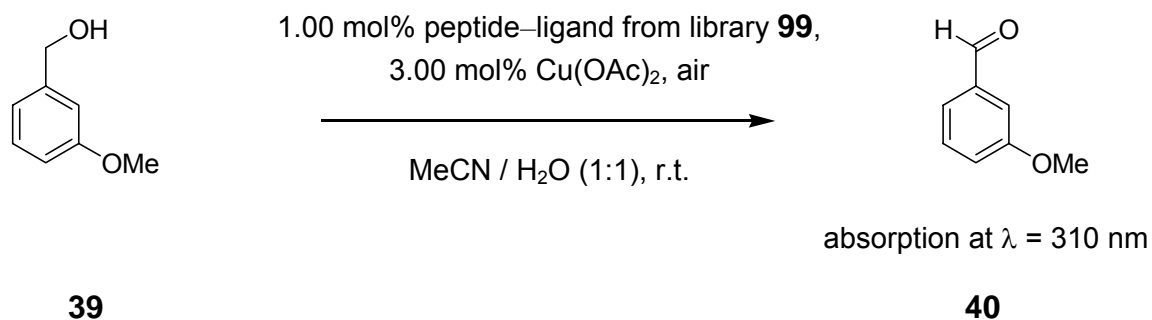
Scheme 5-30: **Assay for the screening of the library of peptide–ligands 99.**[64, 98]

The reaction was followed for 24 hours and some differences in the members of the library were observed (**Table 5-5**, **Figure 3-1**). It is worth to mention that the peptides which did not contain any TOAC residue did not show any catalytic activity (less than 0.5 % yield of 3-methoxybenzaldehyde). The on-bead peptide–ligands containing one TOAC residue showed a low catalytic activity (between 0.5 and 1.5 % 3-methoxybenzaldehyde formed), with the exception of the peptide–ligand where X_1=Tyr, X_2=His, X_3=TOAC and X_4=Tyr (**99-16**) which formed 3 % of oxidation product. The on-bead peptide–ligand containing 2 or 3 TOAC moieties showed the best results with 2.5 to 5.5 % yield. Six peptide–ligands were particularly interesting because of their remarkable catalytic activity (members with a frame in **Figure 5-16**). These are the peptide–ligands **99-09**, **99-18**, **99-27**, **99-63**, **99-72** and **99-75** and they gave more than 3 % of oxidation product.
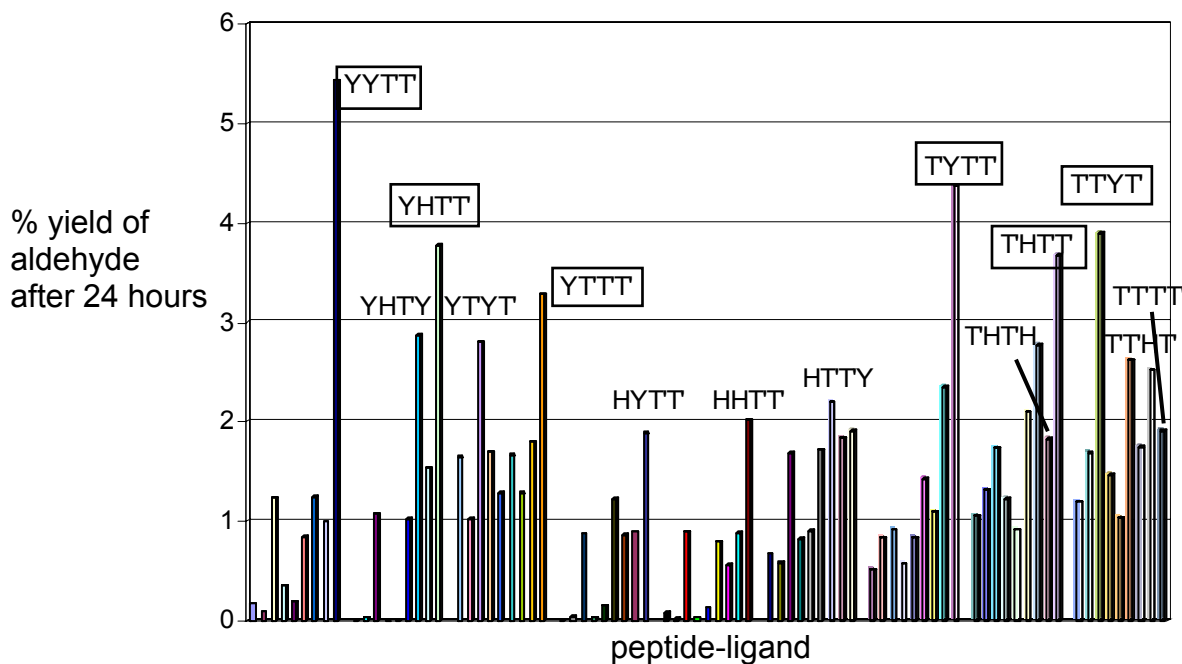
Figure 5-16: Yield of 3-methoxybenzaldehyde after 24 hours in the catalytic aerobic oxidation with the on-bead peptide–ligands from the library 99. From left to right are all the members of the library in the order given in the experimental section with compound 99-01 to the left and compound 99-81 to the right. As an example, YYT'T' corresponds to the peptide–ligands where X_1 = Tyr, X_2 = Tyr, X_3 = TOAC and X_4 = TOAC.

The yields and the turnover numbers obtained with the best members of the library are summarised in **Table 5-5**.

Table 8-9 in the experimental section gives the quantity of 3-methoxybenzaldehyde formed with all the members of the library.

For some members of the library, the catalytical experiment has been reproduced and the yield has been determined by GC analysis. The quantity of 3-methoxybenzaldehyde obtained from GC analysis appears in brackets in **Table 5-5**. The reproducibility of the results shows the reliability of the screening method.

Table 5-5: **Yields of 3-methoxybenzaldehyde after 24 hours in the catalytic aerobic oxidation with the on-bead peptide-ligands from library 99.**
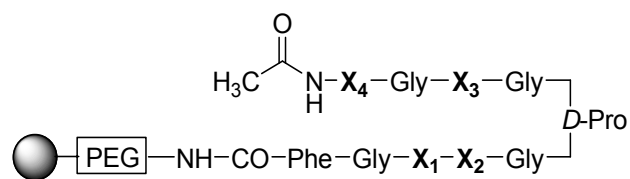
entry	peptide–ligand	peptide sequence[a]				3-methoxybenzaldehyde **40**		
		X_1	X_2	X_3	X_4	[μmol][b]	yield [%][c]	TON[c]
1	**99-09**	Y	Y	T'	T'	21.7 (18.5)	5.4	5
2	**99-16**	Y	H	T'	Y	11.5 (11.9)	2.9	3
3	**99-18**	Y	H	T'	T'	15.1 (17.4)	3.8	4
4	**99-21**	Y	T'	Y	T'	11.2 (13.6)	2.8	3
5	**99-27**	Y	T'	T'	T'	13.1 (11.4)	3.3	3
6	**99-36**	H	Y	T'	T'	7.53 (5.91)	1.9	2
7	**99-45**	H	H	T'	T'	8.07 (9.98)	2.0	2
8	**99-52**	H	T'	T'	Y	8.77 (10.4)	2.2	2
9	**99-63**	T'	Y	T'	T'	17.5 (19.6)	4.4	4
10	**99-71**	T'	H	T'	H	7.29	1.8	2
11	**99-72**	T'	H	T'	T'	14.7 (15.3)	3.7	4
12	**99-75**	T'	T'	Y	T'	15.6 (12.7)	3.9	4
13	**99-78**	T'	T'	H	T'	10.5 (11.6)	2.6	3
14	**99-81**	T'	T'	T'	T'	7.64	1.9	2

[a] Y = tyrosine, H = histidine, T' = TOAC

[b] The values in bracket correspond to the results obtained by GC analysis.

[c] Calculated from the UV results.

In some additional experiments where the catalysis was carried out without copper salt, the product was formed in very little quantity (**Table 5-6**). The same catalytic

experiment was carried out with 3 different peptide-ligands (**99-75**, **99-78** and **99-81**) in the presence or without copper(II) acetate. When the copper salt was used, between 7.64 and 15.6 μmol of 3-methoxybenzaldehyde were formed after 24 hours (entry 1, 3 and 5). When no copper salt was used, less than 1 μmol of 3-methoxybenzaldehyde were formed after 24 hours (entry 2, 4 and 6).
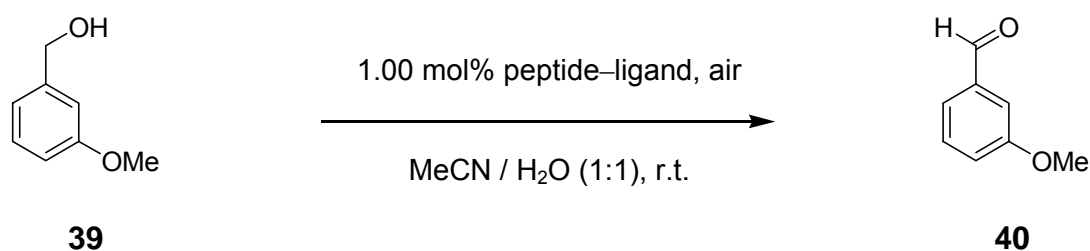
Scheme 5-31: Aerobic oxidation of 3-methoxybenzyl alcohol with a peptide–ligand and without copper salt.

Table 5-6: Comparison of 3 peptide–ligands with and without copper salt in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol.

entry	peptide–ligand	peptide sequence[a]				Cu(OAc)$_2$	aldehyde after 24 hours
		X$_1$	X$_2$	X$_3$	X$_4$	[mol%]	[μmol]
1	**99-75**	T'	T'	Y	T'	3.00	15.6
2	**99-75**	T'	T'	Y	T'	-	0.60
3	**99-78**	T'	T'	H	T'	3.00	10.5
4	**99-78**	T'	T'	H	T'	-	0.50
5	**99-81**	T'	T'	T'	T'	3.00	7.64
6	**99-81**	T'	T'	T'	T'	-	0.50

[a] Y = tyrosine, H = histidine, T' = TOAC

Moreover, the catalysis performed under the same conditions but using soluble TOAC and *L*-Tyr in different proportions did not give as good results as the one obtained when the amino acids were involved in a peptide backbone (**Table 5-7**). Only 0.25 μmol of 3-methoxybenzaldehyde were formed after 24 hours in the background experiment, i.e. without peptide, without amino acid and without copper

salt (entry 1). The experiment with 3 mol% of copper(II) acetate and without presence of peptide or amino acid gave 0.57 μmol of 3-methoxybenzaldehyde after 24 hours (entry 2). Experiments with soluble Fmoc-TOAC (entry 3-6) or soluble *L*-tyrosine (entry 11-14) between 1 and 4 mol% and without copper salt gave less than 1.5 μmol of 3-methoxybenzaldehyde. The oxidation in the presence of copper(II) acetate and Fmoc-TOAC gave a maximum of 5 μmol of 3-methoxybenzaldehyde after 24 hours using 3 mol% of copper and 4 mol% of Fmoc-TOAC (entry 10). The oxidation in the presence of copper(II) acetate and *L*-tyrosine gave less than 2 μmol of 3-methoxybenzaldehyde after 24 hours with 1 to 4 mol% *L*-tyrosine (entry 15-18). Finally, with 3 mol% of copper(II) acetate, 2 mol% of Fmoc-TOAC and 2 mol% of *L*-tyrosine, only 0.25 μmol of 3-methoxybenzaldehyde were formed after 24 hours (entry 19). These values are below the values obtained for the best peptide-copper complexes (more than 10 μmol, **Table 5-5**).
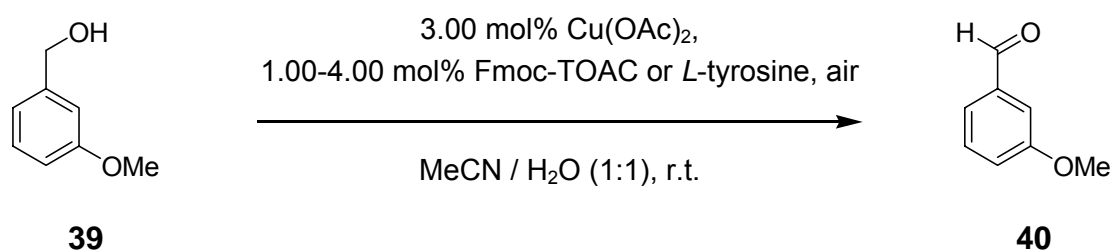
Scheme 5-32: Aerobic oxidation of 3-methoxybenzyl alcohol with soluble amino acids.

Table 5-7: **Catalytic effect of non-peptidic Fmoc-TOAC 43 and *L*-tyrosine on the aerobic oxidation of 3-methoxybenzyl alcohol.**

entry	Cu(OAc)$_2$ [mol%]	Fmoc-TOAC [mol%]	*L*-tyrosine [mol%]	aldehyde after 24 hours [μmol]
1	-	-	-	0.25
2	3.00	-	-	0.57
3	-	1.00	-	0.43
4	-	2.00	-	0.38
5	-	3.00	-	0.42
6	-	4.00	-	1.45
7	3.00	1.00	-	4.23
8	3.00	2.00	-	4.32
9	3.00	3.00	-	4.25
10	3.00	4.00	-	5.05
11	-	-	1.00	0.94
12	-	-	2.00	0.89
13	-	-	3.00	1.05
14	-	-	4.00	0.43
15	3.00	-	1.00	0.25
16	3.00	-	2.00	2.10
17	3.00	-	3.00	0.63
18	3.00	-	4.00	1.82
19	3.00	2.00	2.00	0.25

In an experiment where the reaction was carried out under argon, the product was obtained in a negligible quantity (**Table 5-8**). 15.09 μmol of 3-methoxybenzaldehyde were formed in the catalytic experiment carried out with peptide–ligand **99-18** under

dioxygen (entry 1) while only 0.25 μmol were formed when the experiment took place in argon (entry 2).
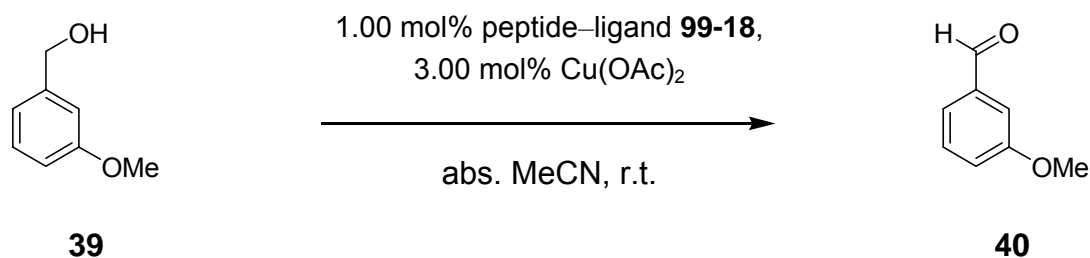
Scheme 5-33: **Oxidation of 3-methoxybenzyl alcohol with peptide–ligand 99-18 under inert atmosphere.**

Table 5-8: **Oxidation of 3-methoxybenzyl alcohol with a peptide–copper complex under air and under inert atmosphere.**

| entry | peptide–ligand | peptide sequence[a] | | | | O_2 | aldehyde after 24 hours |
		X_1	X_2	X_3	X_4		[μmol]
1	**99-18**	Y	H	T'	T'	yes	15.1
2	**99-18**	Y	H	T'	T'	no	0.25

[a] Y = tyrosine, H = histidine, T' = TOAC

In the last experiment with the peptide-ligands, six of them were washed with water, dried and subsequently used in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol (**Table 5-9**). To three of them, copper(II) acetate was added in the solution. Unfortunately, the catalysts were not active anymore since none of the 6 catalysts gave more than 2 μmol of 3-methoxybenzaldehyde after 24 hours.
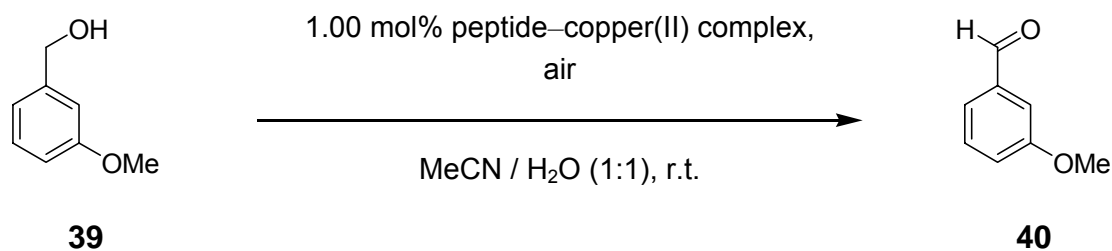
Scheme 5-34: **Recycling of the peptide–copper complexes for the aerobic oxidation of 3-methoxybenzyl alcohol.**

Table 5-9: **Yield of 3-methoxybenzyl alcohol after 24 hours for the second use of the copper catalysts.**

entry	peptide–ligand	Cu(OAc)$_2$ [mol %]	peptide sequence[a]				aldehyde after 24 hours [μmol]
			X$_1$	X$_2$	X$_3$	X$_4$	
1	**99-63**	-	T'	Y	T'	T'	1.30
2	**99-71**	-	T'	H	T'	H	1.50
3	**99-78**	-	T'	T'	H	T'	1.30
4	**99-43**	2.00	H	H	T'	Y	1.00
5	**99-44**	2.00	H	H	T'	H	1.60
6	**99-80**	2.00	T	T	T	H	2.00

[a] Y = tyrosine, H = histidine, T' = TOAC

5.6 Synthesis of one of the members of the library on cleavable resin, cleavage from the resin and catalysis with this peptide-ligand

In order to check the quality of the peptides and to confirm their activity, one member of the library **99** was re-synthesised on cleavable resin (*Wang* resin), analysed and purified by preparative HPLC. The catalytic activity of this soluble peptide-ligand was compared to the activity of its solid-supported equivalent.

The on-bead peptide-ligand **99-71** was chosen as the candidate to be re-synthesised. This choice came from the fact that it was easy to be synthesised. This peptide-ligand was not among the best members of the library.

99-71

101
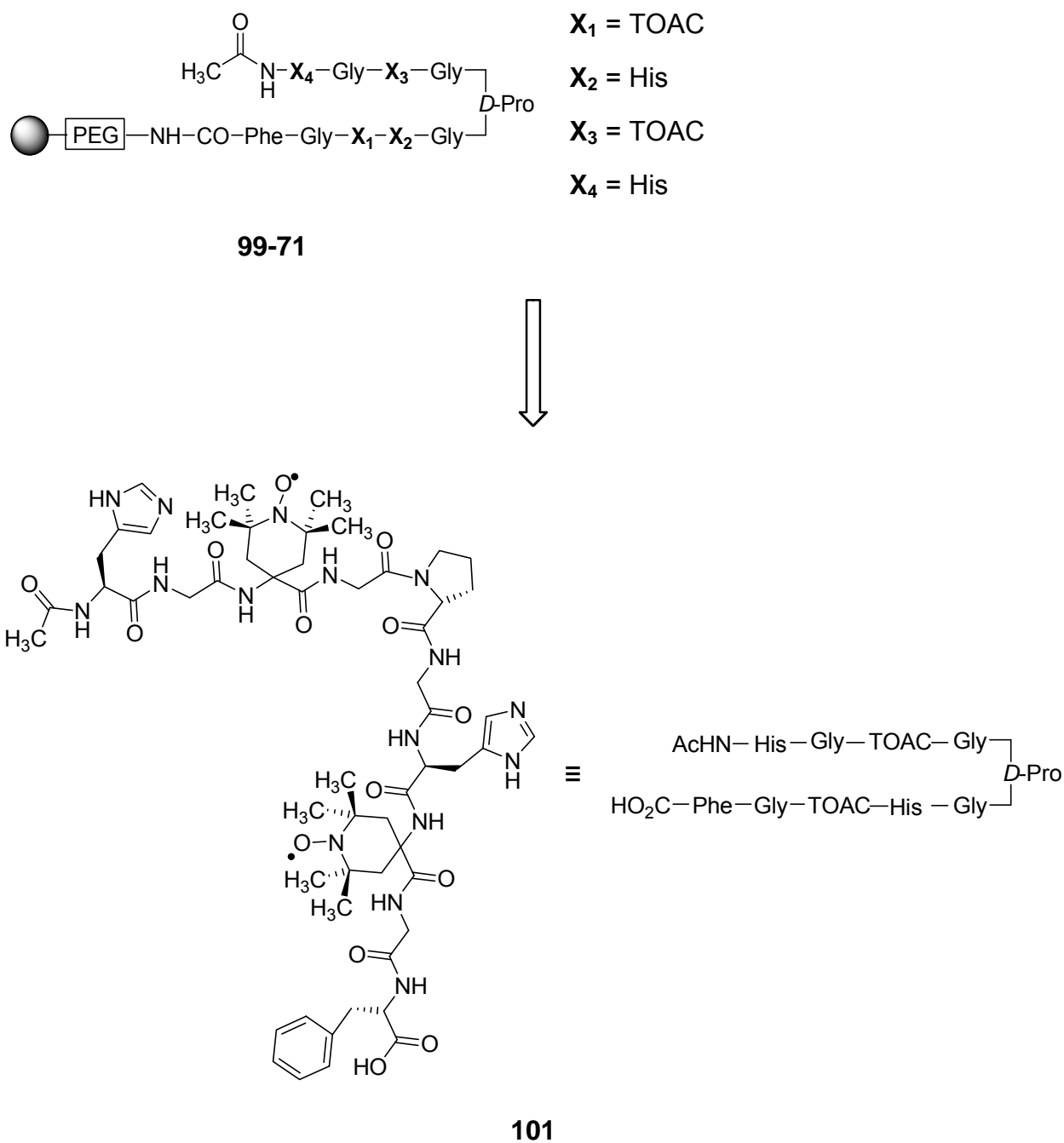
Figure 5-17: On-bead peptide-ligand 99-71 chosen to be re-synthesised and its soluble equivalent 101.

The peptide-ligand **101** was synthesised on TentaGel resin with an acid labile *Wang* linker. The TentaGel resin was used in order to keep the same solid support as the one for the synthesis of the peptide library **99**. In this second synthesis, the linker was the only variable. The TentaGel *Wang* resin **102** used was already loaded with Fmoc-*L*-Phe. Therefore the synthesis started with the cleavage of the Fmoc protecting group (**Scheme 5-35**). By UV measurement of the cleavage solution, the

loading of the resin was determined to be 0.24 mmol/g. The decapeptide was synthesised according to the standard Fmoc/PyBOP strategy.[43] The coupling steps were checked by colour test for free amino functions (NF31 test)[60] and in case of a positive colour test, the coupling was repeated. Finally, the yield of each coupling step was estimated by UV measurement of the cleavage solution. The yield for each coupling step are summarised in **Table 5-10**.

102

1) 20 % piperidine in DMF

2) 2.50 eq Fmoc-Gly-OH, 2.50 eq PyBOP, 3 % NMM in DMF

3) test for free amino functions

4) 20 % piperidine in DMF

5) repetition of the steps 2) to 4) with Fmoc-TOAC-OH, Fmoc-*L*-His(Trt)-OH, Fmoc-Gly-OH or Fmoc-*D*-Pro-OH until completion of the decapeptide

103
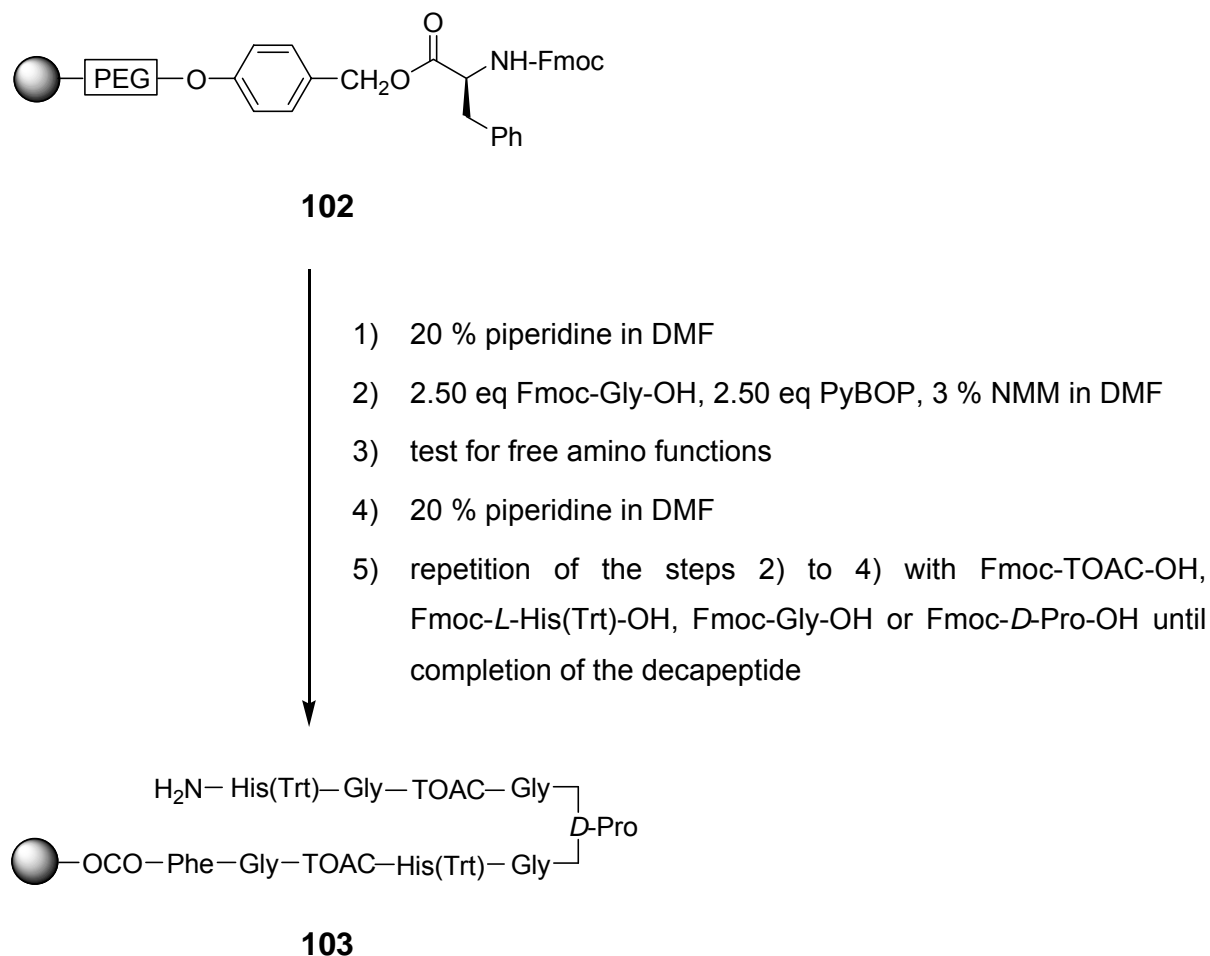
Scheme 5-35: **Synthesis of the decapeptide 103 on TentaGel *Wang* resin loaded with Fmoc-*L*-Phe following the standard Fmoc/PyBOP protocol.**

Table 5-10: **Synthesis of the decapeptide 103 on TentaGel *Wang* resin. Number of coupling steps and yield for each coupling.**

amino acid[a)]	Nr of couplings	yield [%]
Phe	-	100
Gly	1	104
TOAC	1	60
His	3	98
Gly	1	103
D-Pro	1	103
Gly	3	96
TOAC	1	54
Gly	3	71
His	3	72

[a)] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

In the same way as for the synthesis of the peptide-library **99**, the *N*-terminus of the decapeptide **103** was acetylated (**Scheme 5-36**). The completion of the reaction was checked by NF31 colour test.

Treatment of peptide **104** with a mixture of TFA, water and TIS (90 : 5 : 5) released the decapeptide from the resin. The side chain protecting groups were removed at the same time. The decapeptide **101** was released as the free carboxylic acid.

H₂N— His(Trt)—Gly— TOAC— Gly⌐
                                    D-Pro
⬤—OCO—Phe—Gly—TOAC—His(Trt)— Gly⌐

103

1) Ac₂O, 3 % NMM in DMF

2) test for free amino functions

H₃C—(C=O)—N(H)— His(Trt)—Gly— TOAC— Gly⌐
                                            D-Pro
⬤—OCO—Phe—Gly—TOAC—His(Trt)— Gly⌐

104

90 % TFA, 5 % H₂O, 5 % TIS

AcHN— His—Gly— TOAC— Gly⌐
                            D-Pro
HO₂C—Phe—Gly—TOAC—His — Gly⌐

101
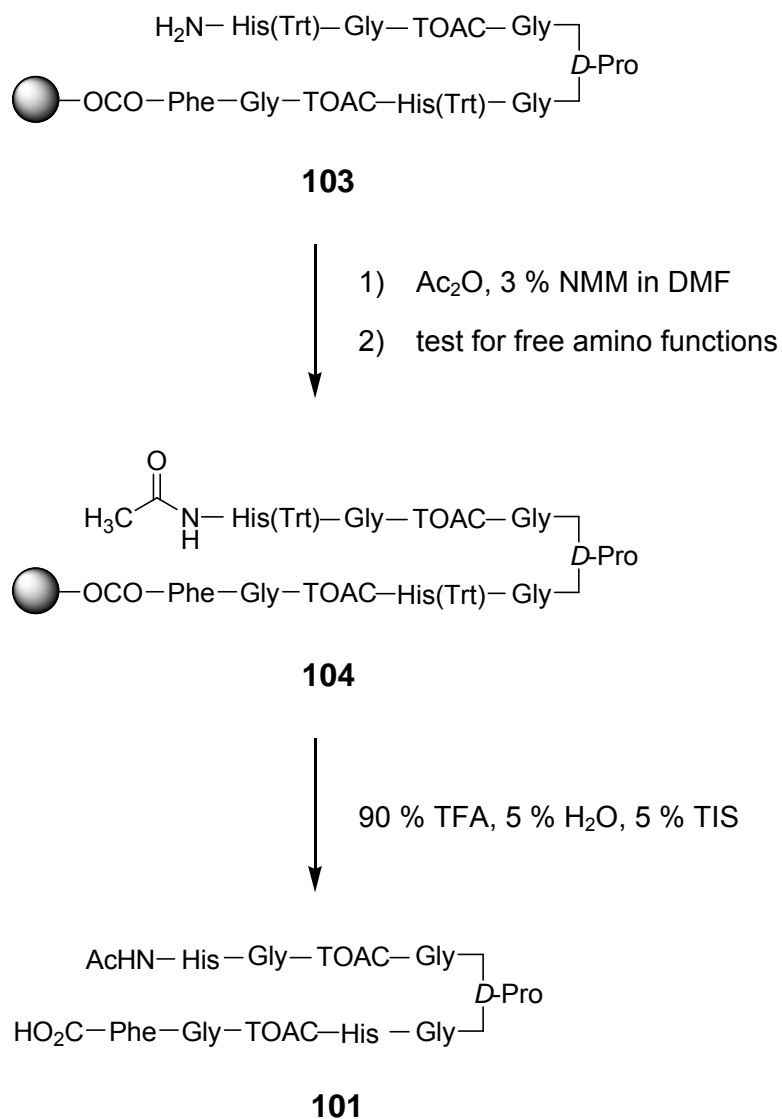
Scheme 5-36: Acetylation of the *N*-terminus of peptide 103 and cleavage from the *Wang* linker.

The decapeptide precipitated by addition of diethylether to the filtrate. From 290 mg of product obtained, 50 mg were purified by preparative HPLC using a reverse phase column (RP-18) and a mixture of water, acetonitrile and acetic acid (90:10:0.5). 38 mg of product were obtained.

Even after purification, the UV trace obtained by LC–MS analysis did not show a clean product (**Figure 5-18**). This chromatogram demonstrated that the decapeptides were present as a mixture of compounds **101**, **105**, **106** and **107**, i.e. the decapeptides with the TOAC moieties in the form of nitroxyl radical or hydroxylamine.
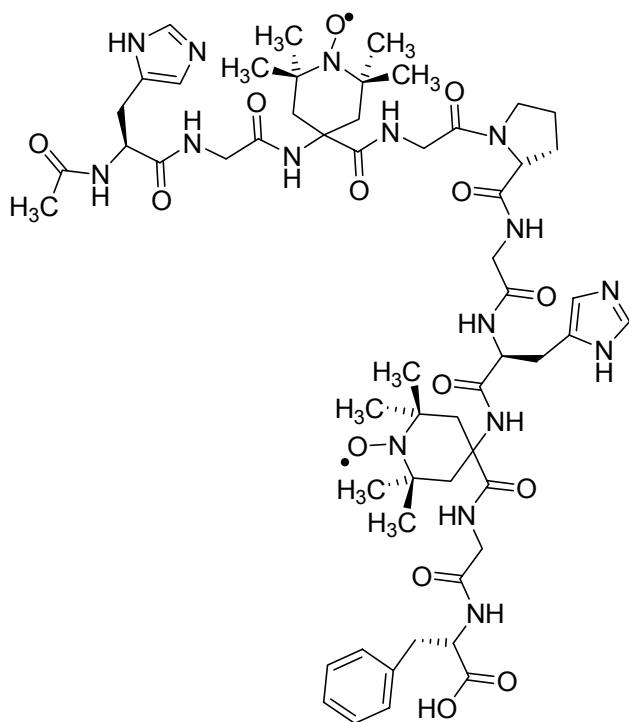
101

m/z 1201
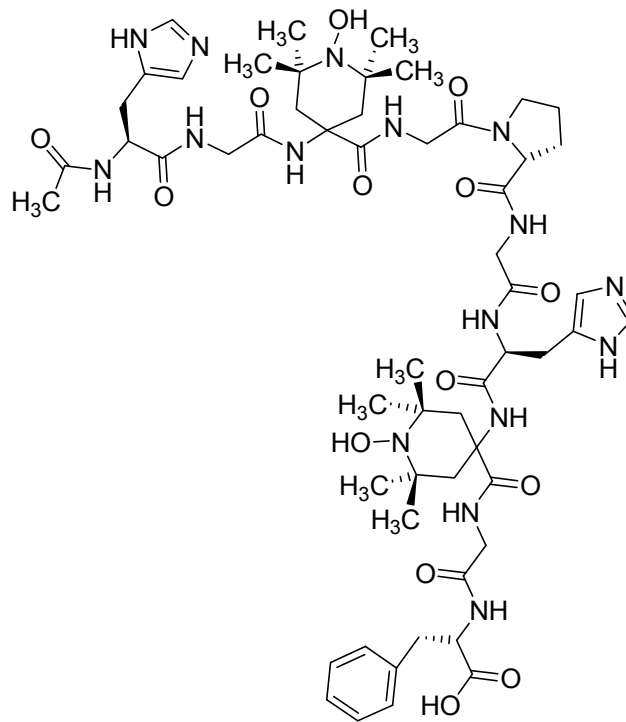
105

m/z 1203
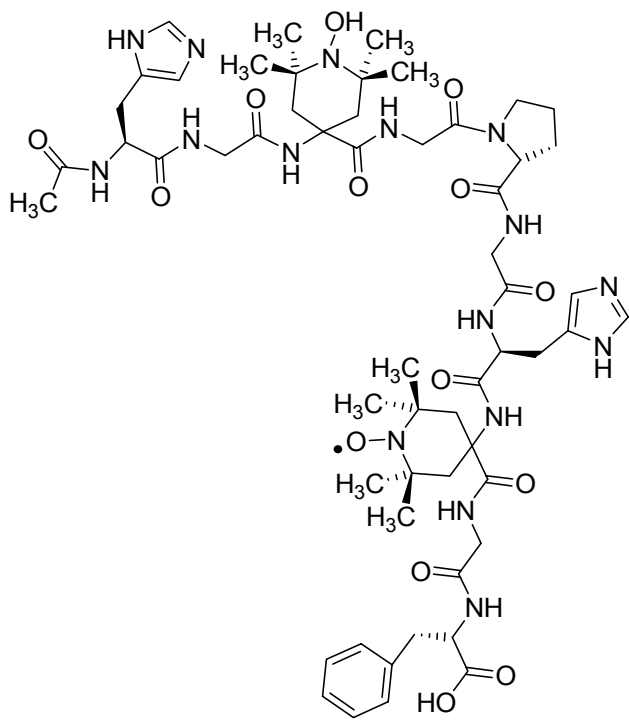
106

m/z 1202
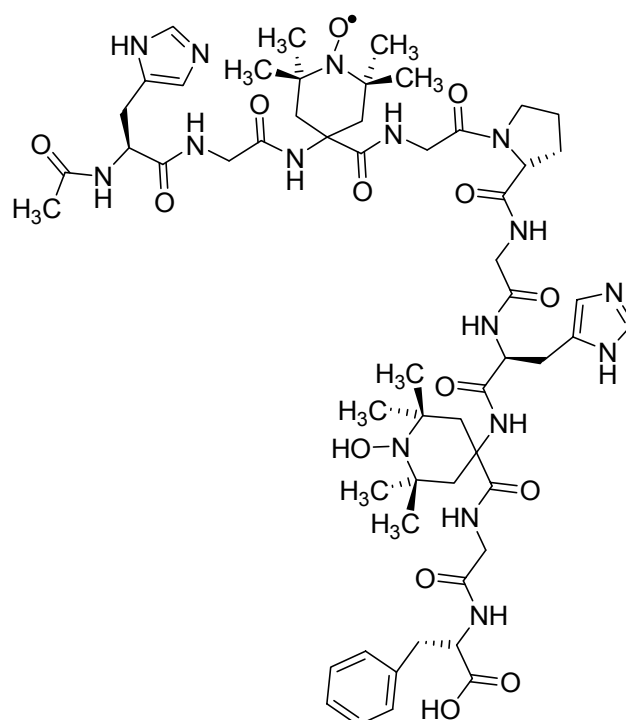
107

m/z 1202
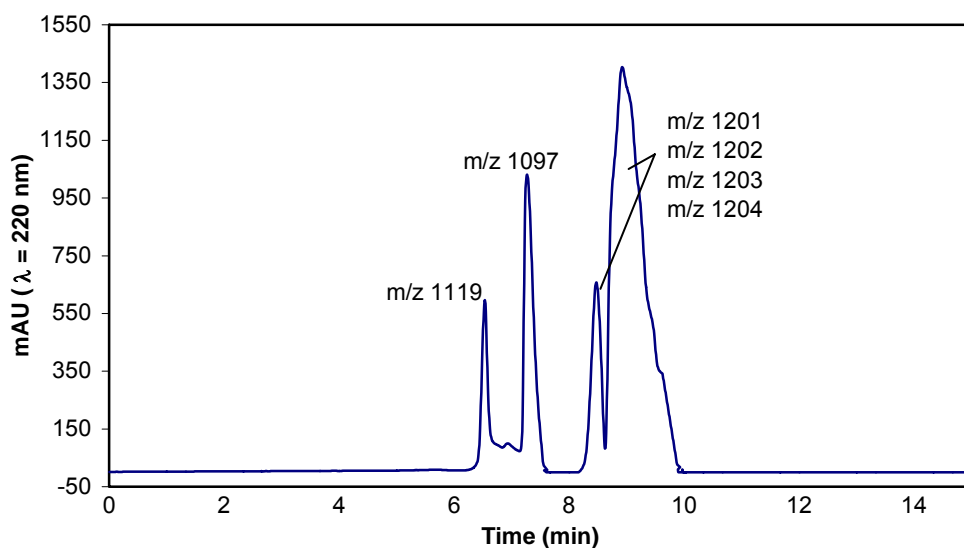
Figure 5-18: **Baseline corrected LC–MS diagram of the product obtained after purification by preparative HPLC (column: RP-18, mobile phase: MeCN : H$_2$O : AcOH = 90 : 10 : 0.5).**

The product which was obtained after purification by preparative HPLC (peptide–ligands **101**, **105**, **106** and **107**) was used in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol, following the same protocol as with the on-bead peptide-ligands (**Scheme 5-37**). After 24 hours, 14.3 μmol of aldehyde **40** were formed. This value was higher than the one obtained with the corresponding on-bead peptide-ligand which was equal to 7.29 μmol.
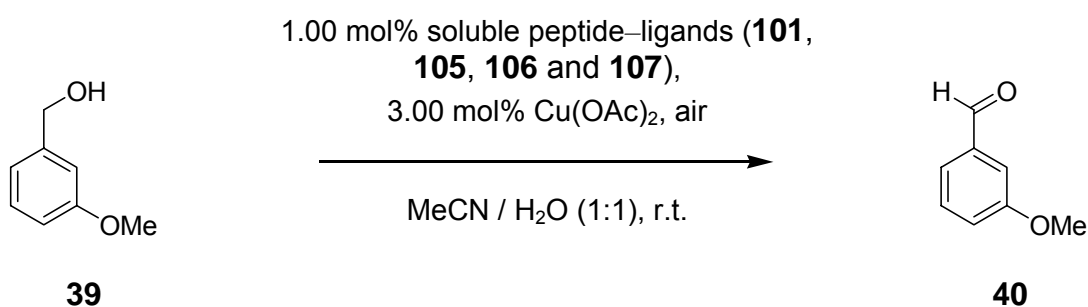
Scheme 5-37: **Catalytic aerobic oxidation of 3-methoxybenzyl alcohol with the soluble peptide–ligands 101, 105, 106 and 107.**

Table 5-11: Catalytic aerobic oxidation of 3-methoxybenzyl alcohol with the soluble peptide–ligands 101, 105, 106 and 107. Comparison with the on-bead peptide–ligand 99-71.

entry	peptide–ligand	peptide sequence[a]				aldehyde after 24 hours
		X_1	X_2	X_3	X_4	[µmol]
1	**99-71** (on-bead)	T'	H	T'	H	7.29
2	**101**, **105**, **106** and **107** (soluble)	T'	H	T'	H	14.3

[a] Y = tyrosine, H = histidine, T' = TOAC

5.7 Synthesis of a resin-supported low molecular weight ligand 66, complexation with copper and catalysis

The tosylamide ligand **66** was synthesised on a solid phase.[89] Chlorosulfonated polystyrene resin (PS-TsCl) **69** was used as a solid support. Starting from this polystyrene-bound tosyl chloride, ligand **66** was synthesised in two steps. Using the advantage of solid-phase synthesis, the reagents were used in excess in order to bring the reactions to completion.

The synthesis was carried out in a *Merrifield* flask, allowing filtration of the solution after reaction and easy isolation of the polystyrene-bound ligand.

In the first step, the PS-TsCl resin **69** was reacted with an excess of ethylenediamine in dichloromethane (**Scheme 5-38**).[117] In order to check the completion of the reaction, a NF31 colour test was carried out on the starting material and on the product. When treated with the NF31 solution and washed, the starting beads remained colourless. On the other hand, treatment of the product **67** with the NF31 solution and washing gave red coloured beads, indicating the presence of free amino functions on the product.[60] Since all the beads had a uniform red colour, it was assumed that the mono-tosylated amine **67** was obtained in quantitative yield.

69

= 1 % cross-linked polystyrene resin
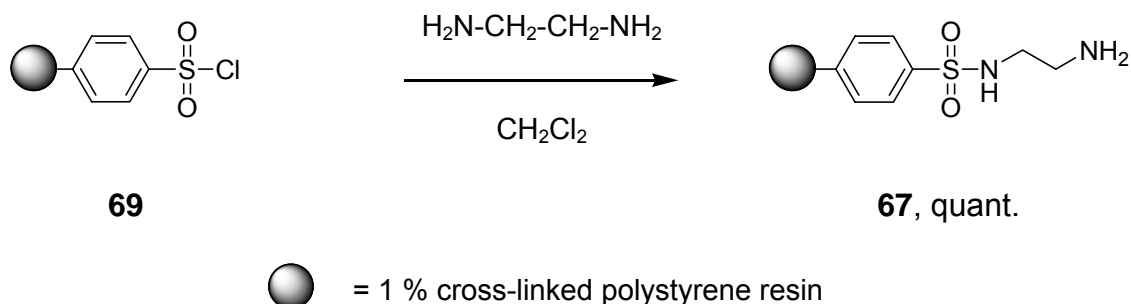
Scheme 5-38: Synthesis of the resin-supported tosylamide 67.[117]

The monotosylated amine **67** was reacted with an excess of 2,4-di-*tert*-butyl-salicylaldehyde **68** to give the on-bead ligand **66** in a quantitative yield.[118] The quality of the on-bead product **66** was checked by HR-MAS NMR.

68

67

66, quant.
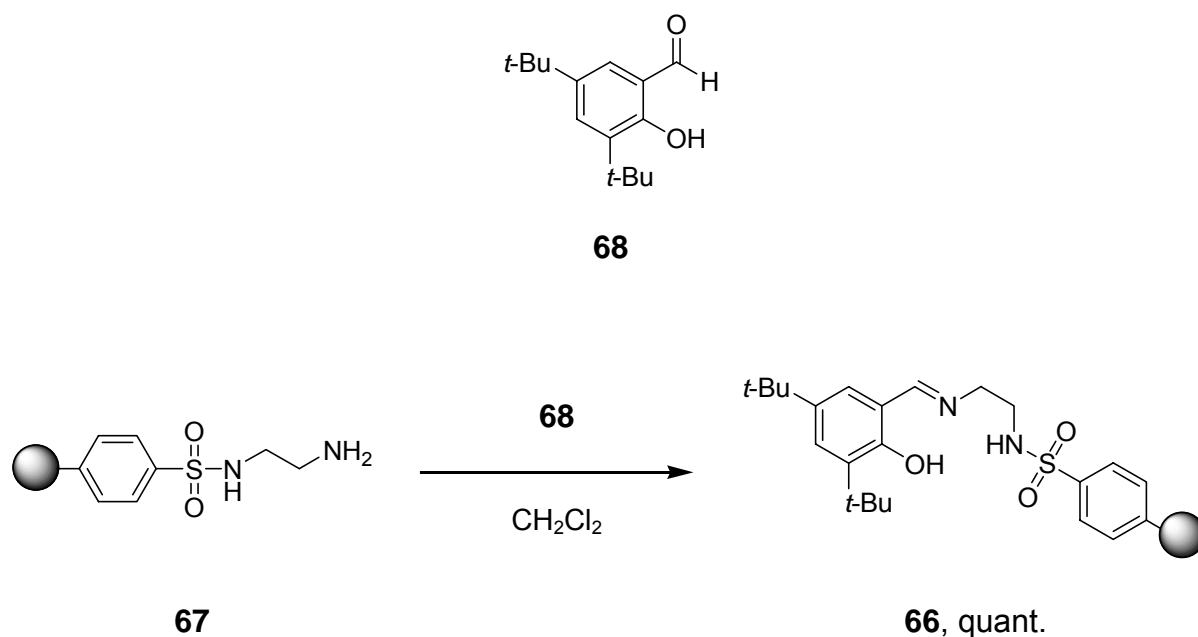
Scheme 5-39: Reaction of the monotosylated ethylenediamine ligand 67 with 3,5-di-*tert*-butyl-2-hydroxybenzaldehyde 68.[118]

The copper complex **65** was formed by reaction of ligand **66** with 1 equivalent of copper(II) acetate in the presence of potassium hydroxide and methanol (**Scheme 5-40**).[90] The observation of the beads under the microscope before and after incubation with copper(II) acetate revealed different colours (**Figure 5-19**). Indeed, the beads supporting ligand **66** had a yellow colour while the one supporting the

copper complex **65** appeared green. The green colour was uniform, which indicated a quantitative complexation of the ligand with copper.
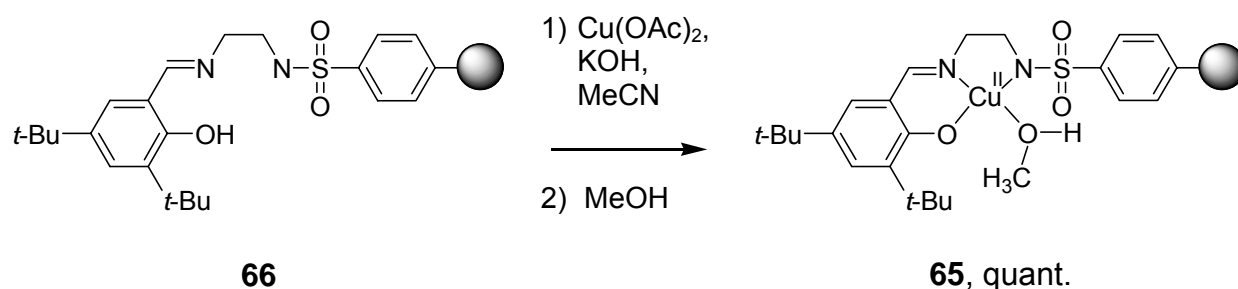
Scheme 5-40: **Complexation of the resin-supported tosylamide 66 ligand with copper(II).**[90]
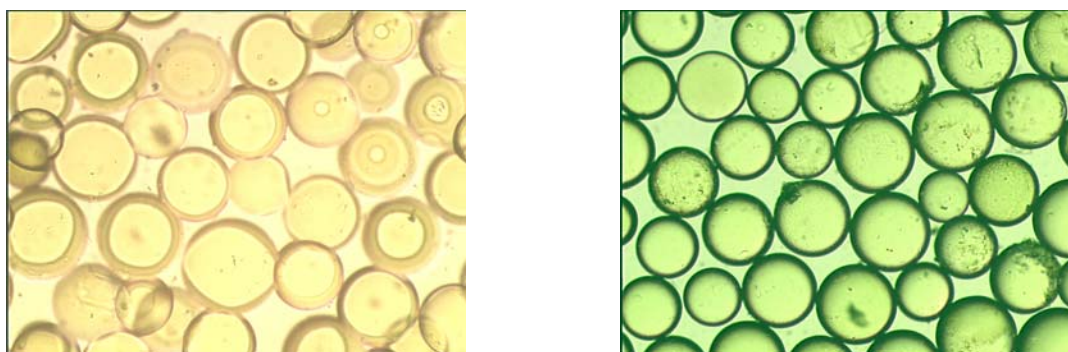
Figure 5-19: **Resin beads of ligand 66 (left) and resin beads of the copper complex 65 (right).**

Ligand **31**, which is the soluble equivalent of ligand **66**, has been synthesised by *Bulat*.[89] His results have shown that the 1:1 copper(II)-ligand complex **32** is organised in a square planar fashion with a molecule of methanol occupying the fourth position around the copper ion. The complexation of ligand **66** with copper(II) acetate in the presence of methanol probably resulted in complex **65** which has a structure similar to the one of complex **32**.
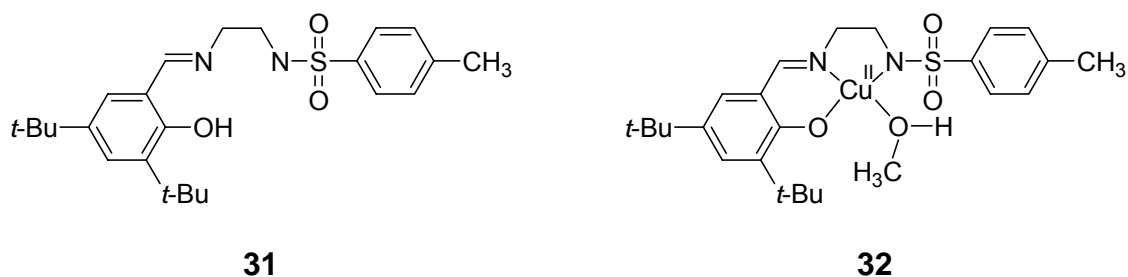
Figure 5-20: Ligand 31 by *Bulat* and its copper complex 32.

The catalytic activity of ligand **66** was compared to the one of ligand **31** in the aerobic oxidation of benzyl alcohols (**Scheme 5-41**).
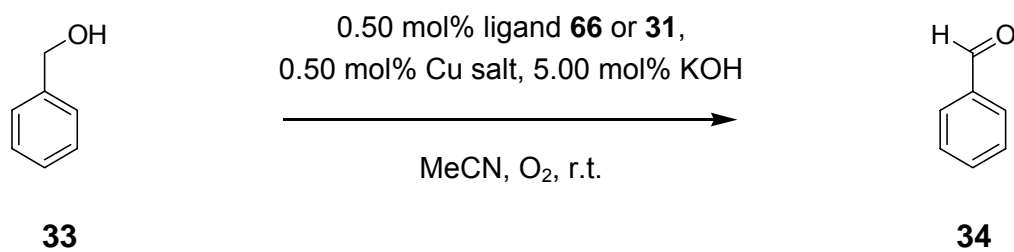
Scheme 5-41: Catalytic aerobic oxidation of benzyl alcohol with the soluble ligand 31 and the solid-supported ligand 66.

The sulfonamide ligand bound to solid-support **66** showed an activity comparable to its soluble equivalent **31** (**Table 5-12**). With 0.5 mol% of ligand, 0.50 mol% of copper(I) chloride and 5.00 mol% of potassium hydroxide, the soluble ligand **31** gave 19 % yield of benzaldehyde (entry 4) while the on-bead ligand **66** gave 18 % of product after 3 hours (entry 2). When copper(II) acetate was used instead of copper(I) chloride, the results were similar: 17 % of benzaldehyde were formed with the soluble ligand **31** (entry 3) and 18 % with the on-bead ligand **66** (entry 4).

Some control experiments were also carried out. In the absence of copper salt (entry 5), only 2 % of product were formed. The catalytical experiment which was carried out in the presence of copper salt and base and without a ligand (entry 6) gave 4 % of benzaldehyde after 3 hours. When the experiments were carried out in the absence of a base, no more than 1 % of benzaldehyde was obtained (entry 7, 8,

9 and 11). Finally, the base did not catalyse the oxidation of benzyl alcohol since only 1 % of benzaldehyde was formed in the absence of copper salt and ligand (entry 10).

Table 5-12: **Yields and turnover after 3 hours for the catalytic aerobic oxidation of benzyl alcohol with ligands 66 and 31.**

entry	ligand	[mol%]	copper salt	[mol %]	yield [%]	TON
1	**66**	0.50	Cu(II)(OAc)$_2$	0.50	18	36
2	**31**	0.50	Cu(II)(OAc)$_2$	0.50	17	34
3	**66**	0.50	Cu(I)Cl	0.50	18	36
4	**31**	0.50	Cu(I)Cl	0.50	19	38
5	**66**	0.50	-	-	2	4
6	-	-	Cu(II)(OAc)$_2$	0.50	4	8
7[b)]	**66**	0.50	Cu(II)(OAc)$_2$	0.50	1	2
8[b)]	**66**	0.50	-	-	1	2
9[b)]	-	-	Cu(II)(OAc)$_2$	0.50	1	2
10	-	-	-	-	1	2
11[b)]	-	-	-	-	1	2

[a)] Benzoic acid was not formed.

[b)] The reaction was carried out without potassium hydroxide.

After 3 hours the catalysis stopped. This phenomenon is similar to the one observed with ligand **31**. Similarly, the appearance of a brown precipitate was observed in the reaction flask. This precipitate was attributed to the formation of copper oxide (**Figure 2-1**).[89]
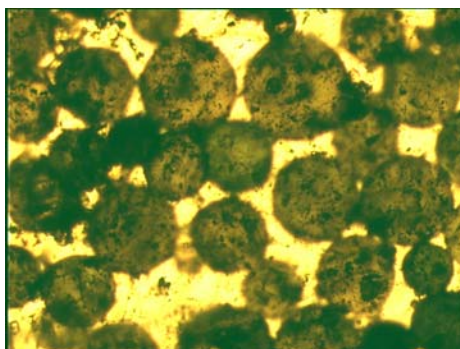
Figure 5-21: Resin beads supporting ligand 66 after catalysis.

The catalytic activity of the soluble and solid-phase bound sulfonamide ligands were compared in the aerobic oxidation of 3-methoxybenzyl alcohol (**Table 5-13**).
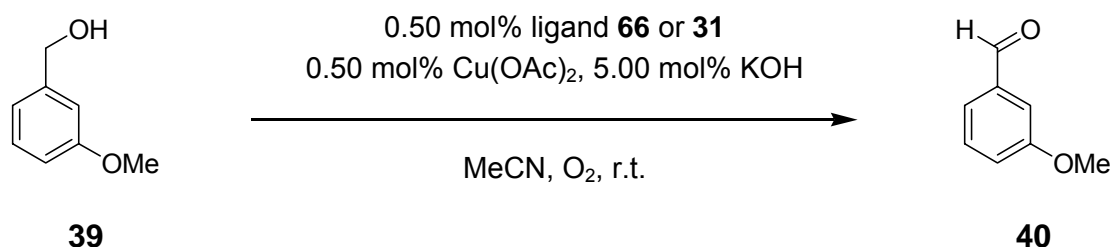
Scheme 5-42: Catalytic aerobic oxidation of benzyl alcohol with the sulfonamide ligands: soluble 31 and bound to solid phase 66.

With 0.50 mol% of ligand, 0.50 mol% of copper(II) acetate and 5.00 mol% of potassium hydroxide, 3-methoxybenzaldehyde was formed in a yield of 16 % with the on-bead ligand **66** (entry 1) and in 13 % yield with the soluble ligand **31** (entry 2).

Table 5-13: Yield and turnover after 3.5 hours for the catalytic aerobic oxidation of 3-methoxybenzyl alcohol with ligands 66 and 31.

entry	ligand	[mg]	[mol%]	% yield in 3-methoxybenzaldehyde[a]	TON
1	**66**	9.70	0.50	16	32
2	**31**	6.00	0.50	13	26

[a] 3-Methoxybenzoic acid was not formed.

Figure 5-22 shows a comparison of the two ligands **31** and **66** in the formation of 3-methoxybenzaldehyde over 4 hours. The solid-supported ligand **66** has an activity comparable to the soluble ligand **31**.
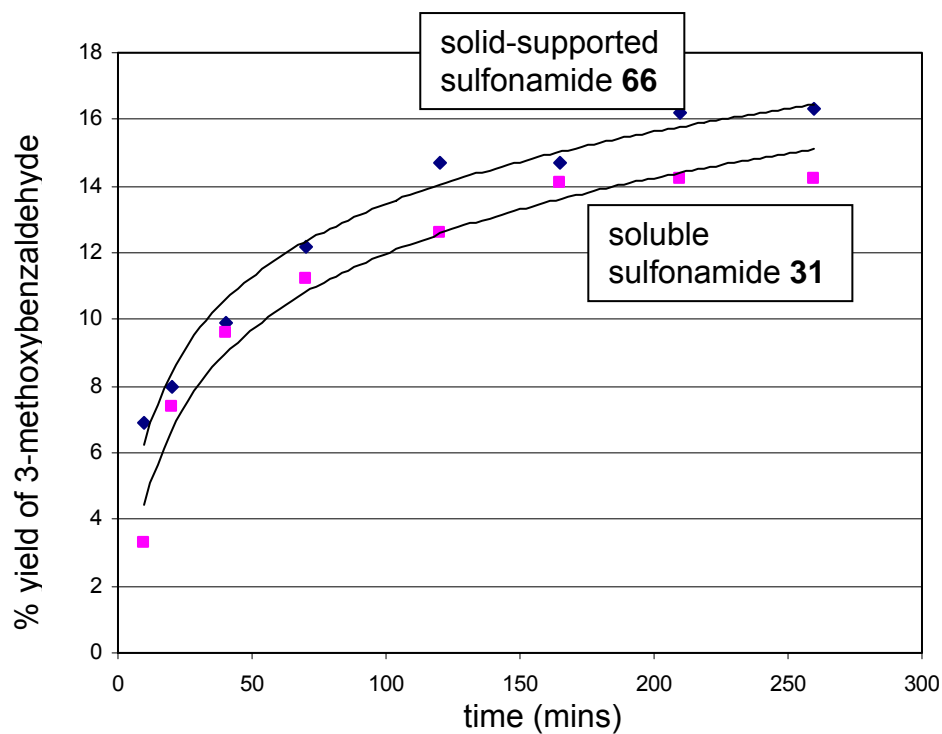
Figure 5-22: **Formation of 3-methoxybenzaldehyde by catalytic aerobic oxidation with ligand 31 and 66.**

6   Discussion

6.1   Towards the synthesis of the unnatural amino acid
Fmoc-*mod*-Cys(MOM)-OH 46

The two amino acids **47** and **53**, *N*-Boc and *N*-Cbz protected respectively, were obtained. Towards the synthesis of the *N*-Fmoc protected amino acid **46**, either the Boc protecting group of **47** or the Cbz protecting group of **53** should be cleaved. In this work, it was not possible to cleave the Boc protecting group without release of the MOM protecting group and the Cbz protecting could not be cleaved hydrogenolytically.
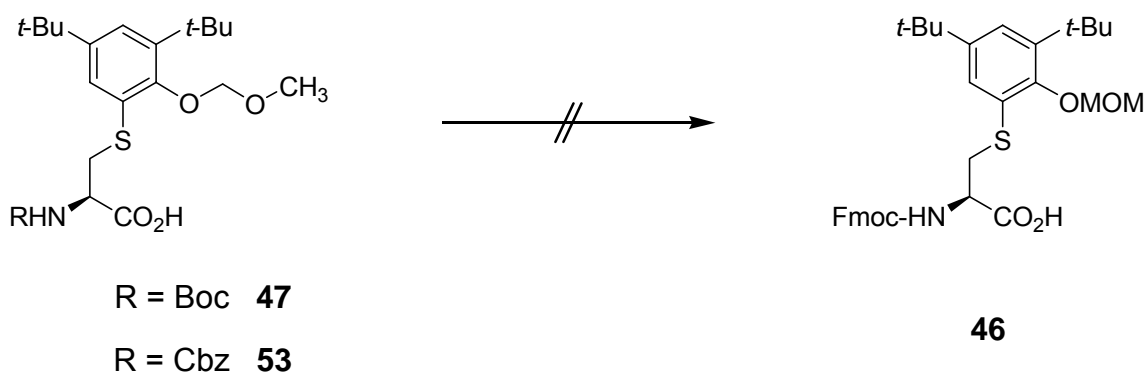
The cleavage of the Boc protecting group of compound **47** occurred together with the cleavage of the MOM protecting group. The reaction was followed by ^{1}H-NMR. These results showed that the Boc protecting group was cleaved before the MOM protecting group since after 10 minutes the Boc protecting group was cleaved while the MOM protecting group was not removed. The cleavage of the MOM protecting group occurred during the work-up of the reaction. Trying to work up the reaction under anhydrous conditions or bringing the pH of the reaction mixture to a basic one before carrying out the work up did not give any better results.

The cleavage of the Cbz protecting group was impossible under classical hydrogenolysis. This is probably due to deactivation of the palladium catalyst by the sulphur atom.

6.2 Synthesis of decapeptides including *mod*-Cys

The two peptide-ligands **78** and **79** were synthesised on amino TentaGel resin.

	X_1 = *mod*-Cys	X_1 = *mod*-Cys
	X_2 = Tyr	X_2 = His
	X_3 = His	X_3 = His
	X_4 = His	X_4 = Tyr
	78	**79**
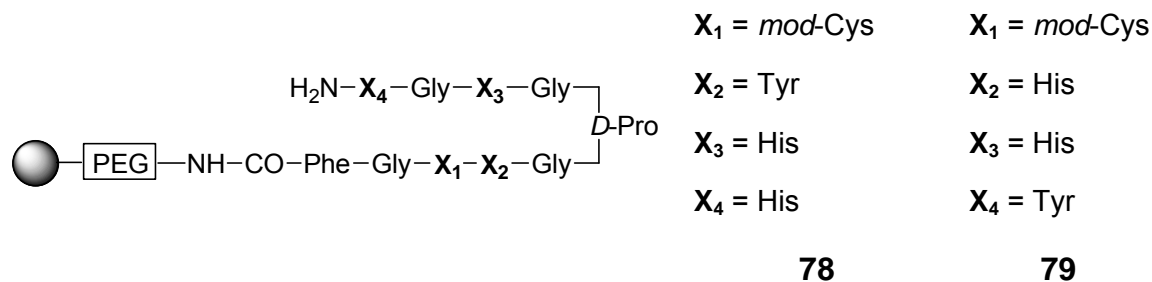
The Boc-*mod*-Cys(MOM)-OH amino acid **47** was included in the peptide sequence of **78** and **79**. The peptide synthesis was carried out in an unusual way since some *N*-Fmoc protected amino acids were used (Fmoc-*L*-Phe-OH, Fmoc-Gly-OH, Fmoc-*D*-Pro-OH, Fmoc-*L*-Tyr(t-Bu)-OH and Fmoc-*L*-His(Trt)-OH), together with the *N*-Boc protected *mod*-Cys **47**. According to the colour test carried out during the synthesis, the coupling steps seemed to be quantitative. The results of the UV analysis of the Fmoc cleavage solution were typical for a peptide synthesis (**Table 6-1**). The coupling yields decreased at the end of the peptide chain, which is not unusual. Only the coupling yield of tyrosine after *mod*-Cys in peptide **78** was quite low (61 % yield). This may be due to steric hindrance which does not occur in the coupling of histidine after *mod*-Cys in peptide **79**.

Table 6-1: **Yield for each coupling step in the synthesis of the decapeptides 78 and 79, determined by UV measurement of the cleavage solution.**

peptide **78**		peptide **79**	
amino acid[a)	yield [%]	amino acid[a)	yield [%][c)
Phe	100	Phe	100
Gly	94	Gly	94
mod-Cys	n.d.	*mod*-Cys	n.d.
Tyr[b)	61	His[b)	100
Gly	78	Gly	89
D-Pro	89	*D*-Pro	94
Gly[b)	94	Gly[b)	111
His	94	His	94
Gly	50	Gly	67
His	44	Tyr	61

[a) All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

[b) The coupling step was repeated after observation of a positive colour test.

[c) The values are relative to the first value which was fixed to 100 %.

For the coupling of the *mod*-Cys amino acid **47**, the phenol function was protected by a MOM protecting group. After the peptide coupling of this amino acid, the Boc protecting group was removed before carrying out the next step of the peptide synthesis. It is very probable that the MOM protecting group was cleaved together with the Boc protecting group during the treatment with TFA. The free phenol could give rise to some branched peptides since the next coupling step could happen at the amino or at the hydroxyl function (**Figure 6-1**).
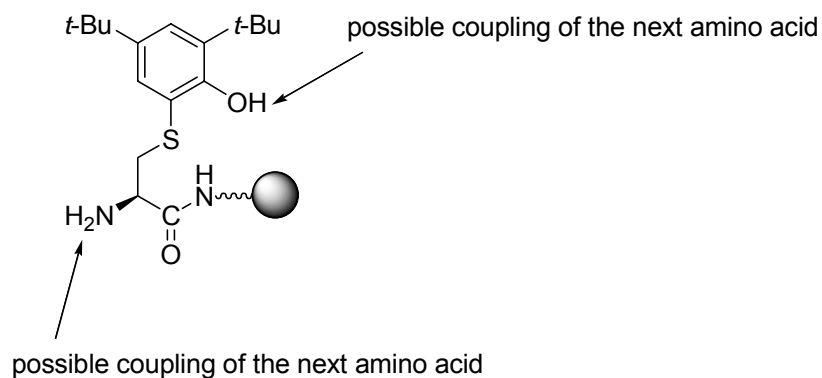
Figure 6-1: **Formation of branched peptides.**

However this did not happen. Indeed, the coupling yields of the amino acids introduced after *mod*-Cys were never higher than 100 % relative to the first amino acid coupling (**Table 6-1**). Indeed, a coupling at the hydroxyl function and at the amino function would lead to a yield higher than 100 %, while a coupling that would occur only at the hydroxyl function would let the amino function free, and therefore would lead to a positive colour test for free amino functions.

The synthesis of these two decapeptides **78** and **79** showed that it was possible to incorporate the *mod*-Cys amino acid in a peptide sequence.

After incubation of the solid-supported decapeptides **82** and **83** with 1 equivalent of copper(II) acetate, the resin-beads observed under the microscope were of a blue–green colour (**Scheme 6-1**). This colour indicated that a peptide–copper complex was formed. It was also shown that acetylated amino TentaGel resin did not bind to copper ion. This suggests that the copper ion bound to the peptide. The ratio peptide-ligand/copper of 1:1 suggested a complex similar to the one observed at the active site of GOase (**Figure 6-2**).
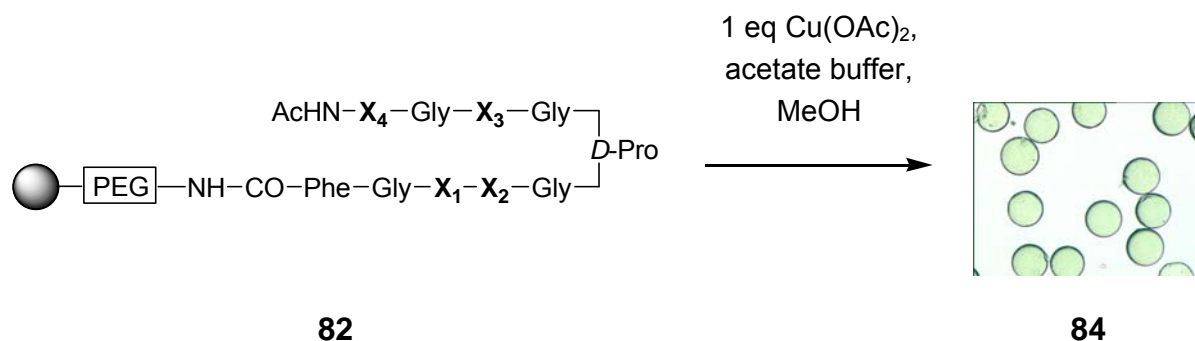
Scheme 6-1: Incubation of decapeptides 82 with 1 equivalent of copper(II) acetate and observation of the resin beads under the microscope.[90]

X$_1$ = *mod*-Cys

X$_2$ = Tyr

X$_3$ = His

X$_4$ = His
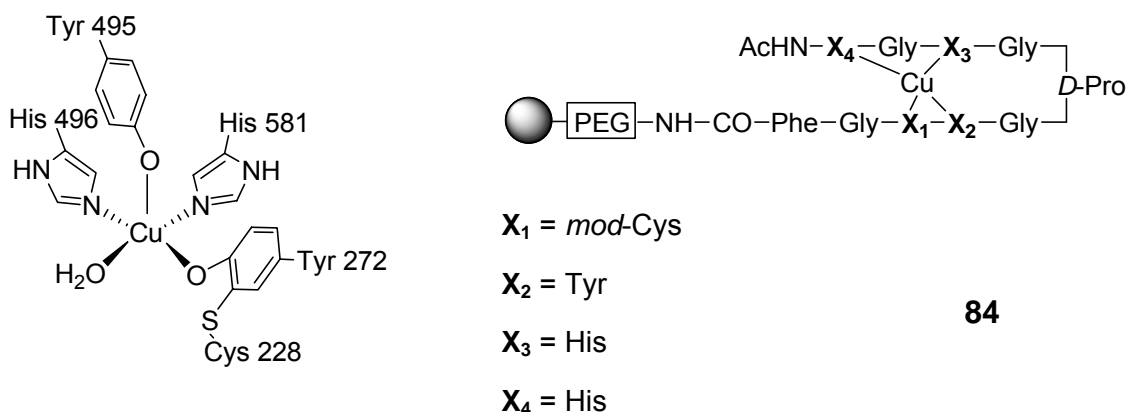
Figure 6-2: Active site of GOase and copper–complex 84.

6.2.1 Catalysis with the peptide–copper complexes 84 and 85

The two peptide–copper complexes **84** and **85** showed catalytic activity in the aerobic oxidation of 3-methoxybenzyl alcohol. Up to 40 turnovers for **84** and 30 turnovers for **85** were obtained after 24 hours, using 0.10 mol % of the catalyst. From these experiments, it was shown that a base was necessary for the catalytic activity. The role of the base was probably to deprotonate the alcohol. Potassium hydroxide led to catalytic activity while triethylamine as a base seemed to deactivate the catalyst. This deactivation could be explained by the binding of the nitrogen base to the copper ion (**Scheme 6-2**).
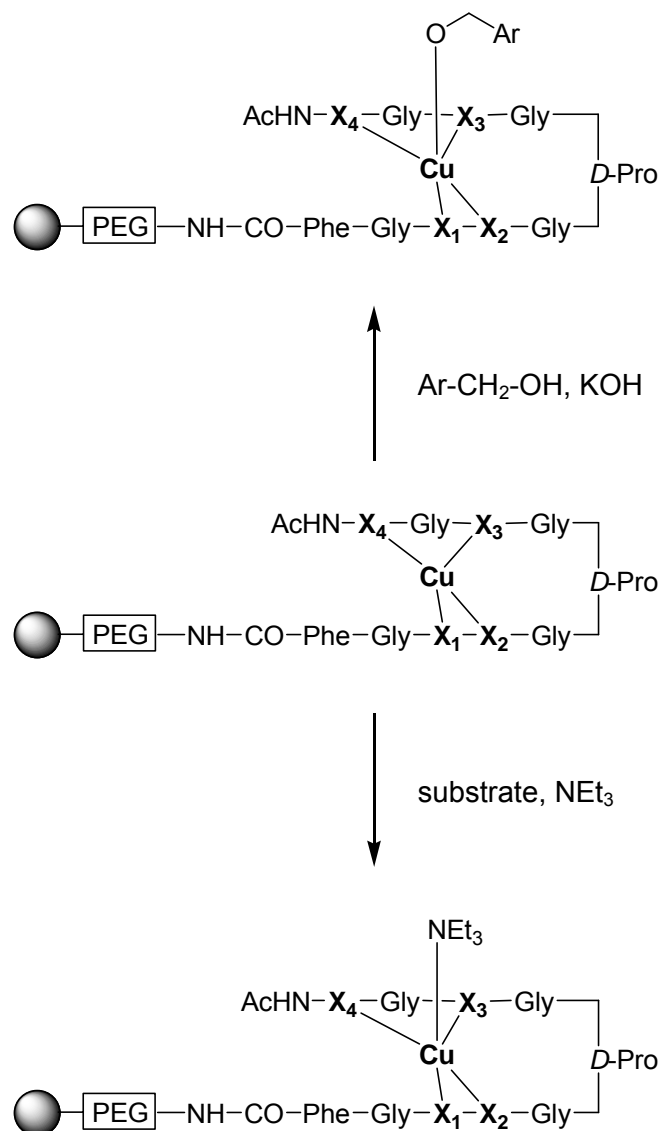
Scheme 6-2: Deactivation of the copper complex by binding of NEt$_3$ to the copper ion.

Copper does not bind to amino TentaGel resin, as shown by the colour of the resin beads which stayed yellow after incubation with copper(II) acetate. It was also shown that amino TentaGel in a copper(II) acetate solution was not active in catalysis. Therefore, the catalytic activity was due to the peptide–copper complexes.

It is also important to note that no brown precipitate was observed in the reaction mixture and that the colour of the beads stayed the same during the catalysis experiment. In comparison to the low molecular weight copper complexes previously synthesised by Bulat,[89] these peptide–copper complexes did not decompose during the catalysis.

These preliminary catalytical experiments were carried out without pre-oxidation of the peptide–copper complexes. Pre-oxidation with potassium hexacyanoferrate(III) or ammonium cerium(IV) nitrate could lead to better results.
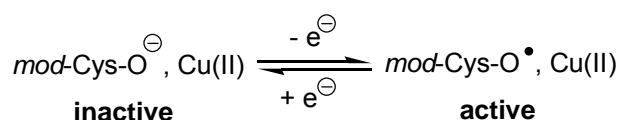
Figure 6-3: Activation of the copper complex by oxidation to the tyrosyl radical.

6.3 Synthesis of Fmoc-TOAC

The Fmoc protection of the amino function of TOAC **41** led to a mixture of the nitroxyl radical **43** and the hydroxylamine **90**.
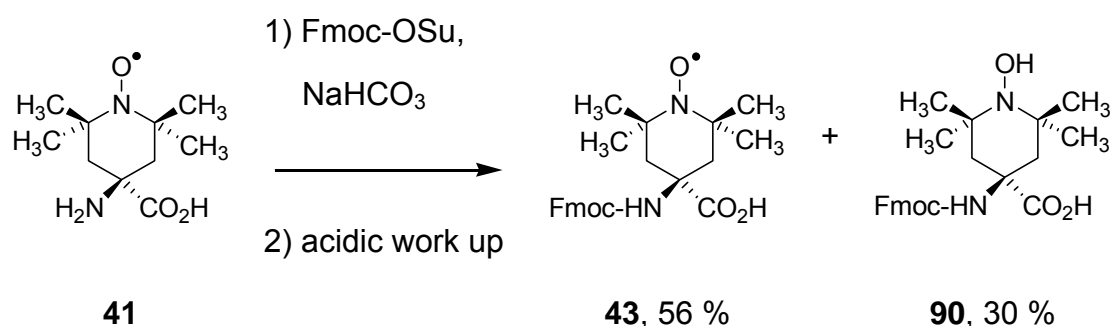
As already suggested by *Toniolo*[114] and by *Jorgensen*,[119] the nitroxyl radical of TOAC was reduced to hydroxylamine in acidic conditions. During the work up, the pH of the reaction mixture was decreased to 3 by the addition of potassium bisulphate. At pH 3, protonation of **43** took place, leading to the formation of **90**.

6.4 Synthesis of the 81-member peptide library of decapeptides 99.

The library of decapeptides **99** was synthesised following the split–mix strategy.

99
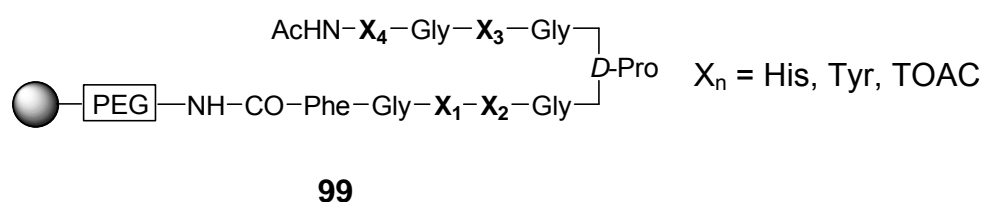
The synthesis was followed by carrying out colour tests for free amino functions at every coupling step. The colour tests suggested a quantitative coupling for each step. Moreover, the peptide synthesis was followed by UV-measurement of the Fmoc cleavage solution which contained the dibenzofulvene–piperidine adduct. The results of the UV-measurement suggested quantitative coupling, except for the coupling of the TOAC amino acid (**Table 6-2**). Therefore, colour test and UV-measurement were in contradiction in the case of the coupling of TOAC. In order to clearify this problem, the tripeptide H_2N-TOAC-Gly-Phe-CO_2H was synthesised on Wang resin, cleaved from the resin and analysed by LC–MS.

Table 6-2: **Coupling yields for the synthesis of the tripeptide on *Wang* resin.**

amino acid	coupling [%]
Phe [a)]	91
Gly	100
TOAC	68

[a)] *L*-amino acid

The analysis of the cleaved product by LC–MS showed the presence of the tripeptide **93**, as well as the hydroxylamine **94**. The dipeptide H_2N-TOAC-Gly-OH **95** was also present in the product mixture. This could be explained by the coupling steps of the first two amino acids. Phe was coupled with in 91 % yield and Gly with in 100 % yield. Therefore 9 % of dipeptide **95** are expected to be present in the mixture of products. It is important to note that the dipeptide H_2N-Gly-Phe-OH **108** was not observed in the reaction mixture, which suggests a quantitative coupling of the TOAC amino acid.

93   **94**   **95**
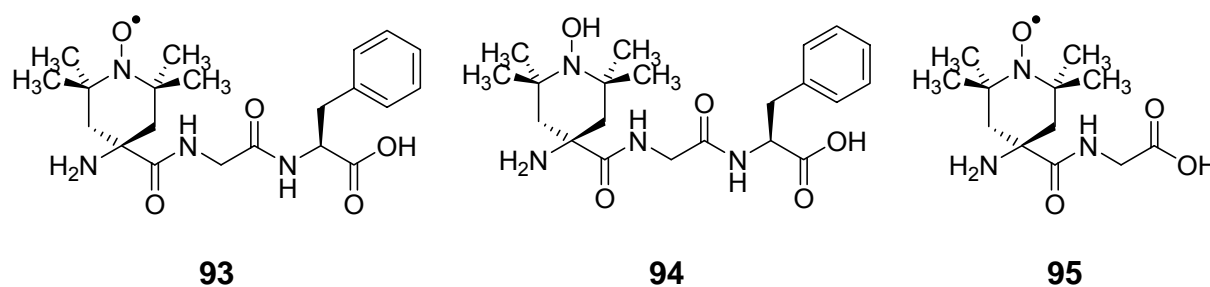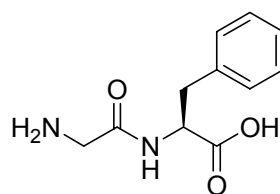
108

The results which were obtained during the synthesis of the tripeptide **93** showed that the use of UV for quantifying the coupling step was not reliable in the case of TOAC. This could be explained by an interaction of TOAC with dibenzofulvene (**Figure 6-4**).
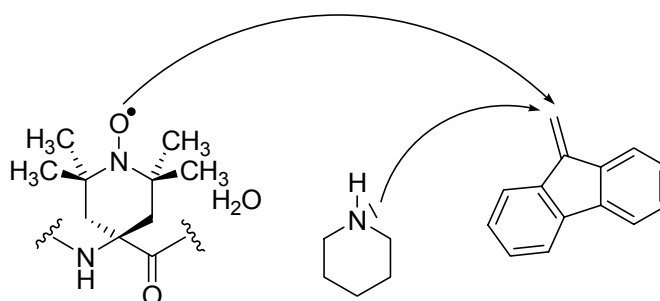
Figure 6-4: Competition between TOAC and piperidine.

The cleavage of the side chain protecting groups of the library of decapeptides **99** was carried out by treatment with TFA. As already observed during the synthesis of Fmoc-TOAC-OH **43** and suggested by *Toniolo*,[114] TOAC nitroxyl radicals are protonated by TFA. Treatment of the decapeptides with an aqueous ammonia solution leads to the regeneration of the nitroxyl radicals. Therefore the nitroxyl radicals were regenerated in order to have the completely activated peptides for the screening of the library.

The complexation of the on-bead peptide-ligands with copper(II) acetate could be visualised by the colour changes of the resin beads. The resin beads carrying the peptide-ligands were pale yellow. After complexation with 1 equivalent of copper(II) acetate, each on-bead copper complex had a particular colour. One copper complex could be distinguished from the others. These various colours observed indicated that the copper ion coordinated at the functional amino acids (tyrosine, histidine and TOAC). Complexation of the copper ion to the resin or to the polyethyleneglycol

moiety can be excluded since the incubation of acetylated amino TentaGel with copper(II) acetate did not resulted in any changes in the colour of the beads. A complexation of the copper ion on the peptide backbone would give the same colour for all the members of the library. The ratio peptide-ligand/copper of 1:1 suggested a complex similar to the one observed at the active site of Goase, i.e. a complex where the copper ion is complexed by one peptide-ligand.

6.5 Screening of the library of decapeptides.

The library of decapeptides **99** was screened for activity in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol. The copper complexes from the library **100** did not show any catalytic activity in acetonitrile/water (1:1).

When the complexes were prepared in situ with 3 equivalents of copper ions relative to the amount of the ligand, the formation of 3-methoxybenzaldehyde could be observed. When the copper salt was added in excess, the solution appeared blue coloured, thus suggesting that the copper excess remained in solution and did not coordinate with the peptide-ligands. The difference in the activity could be explained by the formation of a substrate–copper complex that would be in an equilibrium with the peptide–copper–substrate complex (**Figure 6-5**).
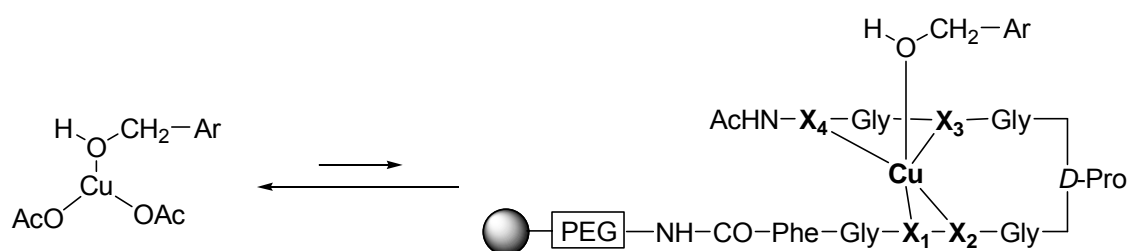
Figure 6-5: **Equilibrium between the soluble substrate–copper complex and the solid-supported peptide–copper–substrate complex.**

Differences in the catalytic activity of the 81 members of the library **99** were observed. After 24 hours, up to 5 % yield were observed for the best catalyst, i.e. the catalyst with the peptide sequence X_1 = Tyr, X_2 = Tyr, X_3 = TOAC and X_4 = TOAC. This corresponds to a turnover frequency of 10^{-4} s^{-1}. 4 % of the aldehyde were formed with the catalysts which had the peptide sequences X_1, X_2, X_3, X_4 = TOAC, Tyr, TOAC, TOAC and TOAC, TOAC, Tyr, TOAC.

Some general trends could be drawn out from the results of the screening. The presence of the TOAC amino acid in the peptide sequence was necessary for catalytic activity. The best catalysts contained two or three TOAC moieties. Better catalytic activity was observed when tyrosine was present in the peptide sequence. Tyrosine was probably acting as a base and deprotonated the substrate (**Figure 6-6**). Histidine was not basic enough to deprotonate the substrate. The catalyst with four TOAC residues did not show a good activity. In this case, there was not a basic moiety for the substrate deprotonation, thus resulting in slower activity. This is consistent with the results of *Sheldon* who showed that in the catalytic system consisting of TEMPO, bipyridine and a copper salt, the addition of a base accelerates the reaction.[9]
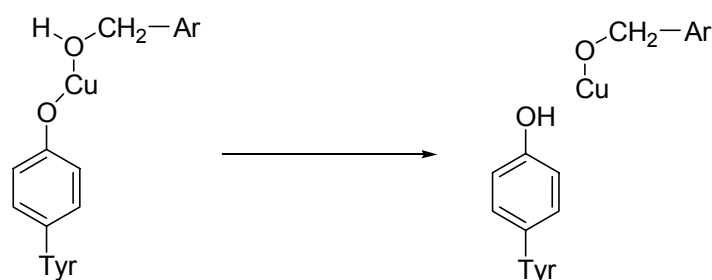
Figure 6-6: Deprotonation of the substrate by the tyrosine moiety.

Moreover, the control experiments have shown that dioxygen and copper were necessary for the oxidation to take place. That means that dioxygen was the primary oxidant and that the oxidation took place at the copper complex. Less aldehyde was formed when soluble amino acids were used instead of the peptides. This showed that the spatial organisation of the copper complexes played a role in the aerobic oxidation.

It was also important to point out that no precipitate was observed in the reaction flask during catalysis, thus suggesting the stability of the catalysts. However the catalyst did not show further activity when it was used for a second time, even when two equivalents of the copper salt were added into the solution.

One of the members of the library was synthesised on a cleavable resin. The activity of the soluble decapeptide in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol was higher than the activity of its on-bead counterpart. While 1.8 % of

3-methoxybenzyl alcohol were converted to the corresponding aldehyde with the on-bead copper complex, 3.6 % of 3-methoxybenzaldehyde were obtained with the soluble copper complex. The soluble decapeptides were purified before catalysis. Even if the product was not clean, it showed that smaller peptide sequences were not present in the reaction mixture. The TOAC moieties in the soluble decapeptide were present as a mixture of nitroxyl radical and hydroxylamines, which did not affect the catalysis. This suggests a catalytic mechanism in which both nitroxyl radical and hydroxylamine functions played a role (**Figure 6-7**). According to this mechanism, a hydrogen atom was abstracted from the alcoholate by the nitroxyl radical, resulting in a ketyl radical. The next step is an electron transfer from the ketyl radical to the copper(II). During this process, the copper(II) was reduced to copper(I) and the aldehyde product was formed, together with the hydroxylamine derivative. The hydroxylamine moiety could finally be re-oxidised to the nitroxyl radical by dioxygen.
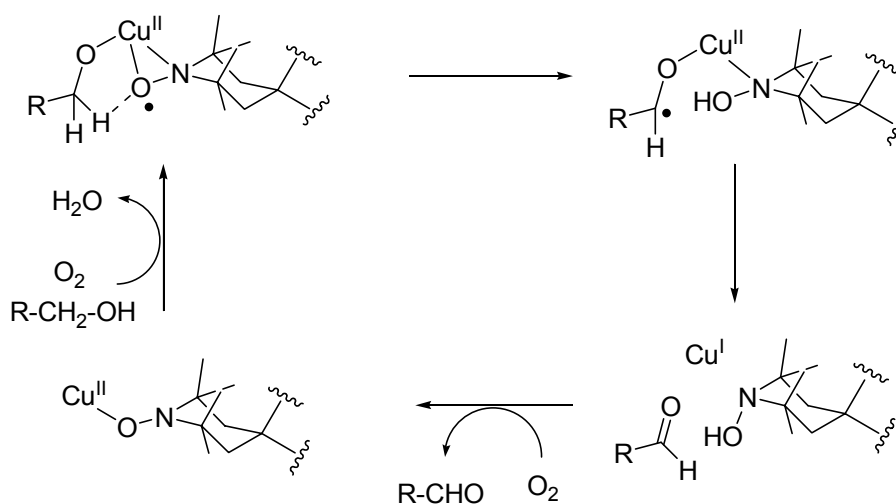
Figure 6-7: Proposed mechanism for the oxidation of the primary alcohol by copper(II) and TOAC moiety based on a mechanism proposed by Sheldon.[9]

6.6 Aerobic oxidation of alcohols with a solid-supported low molecular weight copper complex

The low molecular weight ligand **66** was synthesised on polystyrene resin.
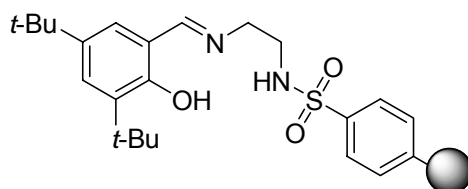
66

Compared to its soluble counterpart, this ligand was more easily synthesised. The 3-step solid phase synthesis gave the product in a quantitative yield without purification. Unfortunately, ligand **66** only showed an activity comparable to the soluble ligand **31**. The catalyst was deactivated after 3 hours. 36 turnover numbers were achieved after 3 hours in the oxidation of benzyl alcohol, which corresponded to a turnover frequency of $3 \cdot 10^{-3} \text{ s}^{-1}$. This activity was higher than the activity of the peptide-ligand which oxidises 3-methoxybenzyl alcohol with a turnover frequency of 10^{-4} s^{-1}.

7   Outlook

7.1   Synthesis of the Fmoc-protected mod-Cys

The Fmoc-protected *mod*-Cys amino acid **46** could not be prepared in this work. In order to synthesise this compound **46**, the protecting-groups strategy should be modified. Since the *O*-MOM protected thiol **55** has proven to be stable and easy to prepare, the changes should concern the other building block, i.e. the lactone. The amino function should be protected with a group that is base-resistant and orthogonal to the MOM protecting group. In this respect, a photolabile protecting group could be a good choice. Particularly, the α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl group is cleaved by irradiation at 282 nm and 276 nm (**Figure 7-1**). The amino function can be protected with such a group by reaction with α,α-dimethyl-3,5-dimethoxybenzyl-oxycarbonyl azide. This protecting group is light sensitive, and thus could be removed without cleaving the sulfide bond and without cleaving the MOM protecting group.
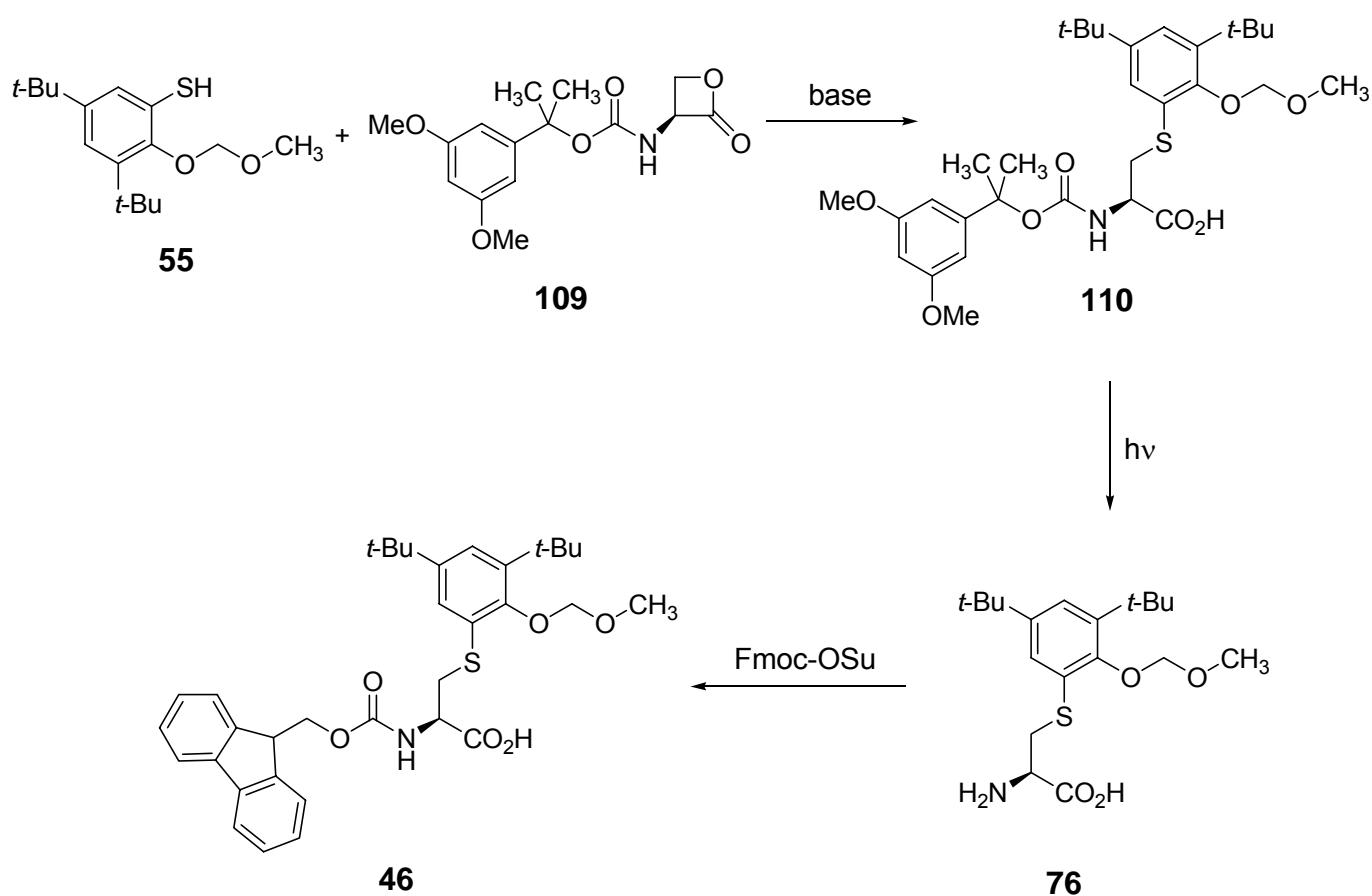
Figure 7-1: Alternative route towards the synthesis of Fmoc-*L-mod*-Cys(MOM)-OH 46.[120]

7.2   Further work concerning the peptide-ligands

The screening of the library of peptide-ligands has shown that some members were more active in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol. Further work could consist in the optimisation of the reaction conditions, i.e. screening of different solvents, addition of a buffer, etc. This could be better done in solution with purified peptides. Screening of a range of substrates should also be done in order to determine the scope of the catalysts.

7.3   Copper complexes for the aerobic oxidation of alcohols

Sheldon has reported on the activity of a bipyridine–TEMPO–copper complex in the catalytic aerobic oxidation of alcohols.[9] The combination of TEMPO- and bipyridine-like functions in a single ligand could result in a more active and stable copper catalyst. *Hoveyda* and co-workers have reported the library of ligand **111**.[121] These peptide–Schiff base ligands were tested in the copper-catalysed allylic alkylation of aryl substituted allylic phosphates, thus demonstrating the ability of these ligands to form copper complexes.
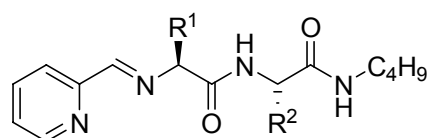
111[121]

A library of such peptide–Schiff base ligands with a pyridine-carboxaldehyde-derived imine could be synthesised on a solid phase (**Figure 7-2**). The peptide sequence should include the TOAC amino acid **41**. Such a structure would allow the variation of the length of the peptide and the synthesis of a diversified library of ligands.
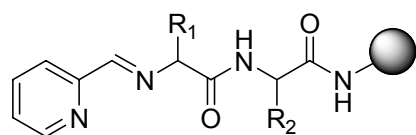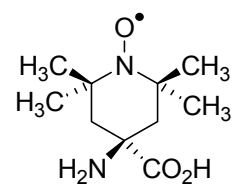
112   **41**

Figure 7-2: **Dipeptide–Schiff base ligand with a pyridine-carboxaldehyde-derived imine.**

8   Experimental part

8.1   General procedures and use of devices

Solvents: All solvents were distilled before use. Anhydrous solvents were first refluxed with the appropriate drying agent and then distilled. As a drying agent, calcium hydride was used for acetonitrile and *n*-hexane, sodium / benzophenone for toluene, tetrahydrofuran and diethylether, magnesium for isopropanol, methanol and ethanol. Absolute *N,N*-dimethylformamide was obtained from a 99 % grade which was refluxed over calcium hydride and distilled.

Substrates: The substrates benzyl alcohol and 3-methoxybenzyl alcohol were distilled prior to catalysis experiments.

Inert gas: Argon from the company *Linde* with a purity of 99.998 % was used after being dried over silica gel desiccant and phosphorus pentoxide (both with colour indicator for humidity), and deoxygenated with a BTS catalyst.

Kugelrohr distillation: Kugelrohr distillations were performed on a *Büchi* B-580 instrument.

Melting points: Melting points were measured with a *Büchi Tottoli* instrument. The values were not corrected.

Infrared spectroscopy (FT-IR): The IR spectra were measured with a *Perkin–Elmer* 1600 Series FT-IR instrument. The samples were measured as potassium bromide or caesium iodide discs. The positions of the bands are given in cm^{-1}, the intensities are given as s = strong, m = medium, w = weak, br = broad and the kind of bands are ν = streching, δ = deformation (oop = out of plane).

Nuclear magnetic resonance (NMR): The NMR measurements of soluble low molecular weight molecules were performed at room temperature on a *Bruker* AC 300, equipped with a narrow bore magnet (Oxford, 7.0 Tesla) and a *Bruker Aspect* 3000 computer. The on-bead measurements were performed on a *Bruker*

Avance DRX 500, equipped with multinuclear HR-MAS probe, a narrow bore magnet (Bruker SpectroSpin, 11.7 Tesla) and a SGI O_2 workstation. The chemical shifts (δ) are relative to the internal standard TMS. The coupling constants (J) are given in Hertz. In the interpretation of the spectra, the abbreviations are the following: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet.

Mass spectrometry (HR-MS): The mass spectra were measured on a *Finnigan* MAT 900 ST instrument.

Elemental analysis (EA): Before analysis, the samples were recrystallised and dried. The elemental analyses were carried out on an *Elementar Analysensysteme GmbH* Vario EL. The percentages of the elements carbon, hydrogen and nitrogen in the samples were determined.

X-Ray analysis: The samples were analysed with a *Nonius* KappaCCD-diffractometer equipped with the program Denzo. The calculations of the structures were performed with the software SheIXS86 and SheIXL93.

Thin layer chromatography (TLC): The TLC analyses were carried out on POLYGRAM Sil G/UV$_{254}$ plates (0.25 mm thick, silica gel with fluorescent indicator) from *Macherey-Nagel*. The spots of products were visualised in UV (λ = 254 nm) or revealed with phosphomolybdic acid.

Column chromatography: For purifications by column chromatography, silica gel or aluminium oxide were used as a stationary phase. The silica gel used was purchased from *Macherey–Nagel* (MN-Kieselgel 60, 0.040–0.063 nm, 230–400 mesh) and the aluminium oxide was purchased from *Fluka* (0.05–0.15 nm, pH 7.0).

Gas chromatography (GC): For the gas chromatography analyses, a *Hewlett–Packard* HP 5890 Series II was used. The device was equipped with a flame ionisation detector and a HP-5 capillary column (95 % methylsiloxane, 5 % phenylsiloxane; 30 m, 0.2 mmID, 0.32 μm thick). Nitrogen was used as a mobile

phase. The chromatograms were analysed with the software HP ChemStation from *Hewlett–Packard*.

Program 1 was used for the detection of benzyl alcohol and its oxidation products, as well as for the detection of 3-methoxybenzyl alcohol and its oxidation products. Diphenylether was used as the internal standard. The program was defined as follows:

> Injector temperature: 250 °C
>
> Detector temperature: 250 °C
>
> Initial temperature: 100 °C for 5 minutes
>
> Gradient of 10 °C/min until 200 °C

Table 8-1: Retention times (τ_r) of the substrates, products and internal standard with GC program 1.

compound	τ_r [min]
benzyl alcohol	5.42
benzaldehyde	4.19
benzoic acid	8.49
3-methoxybenzyl alcohol	9.73
3-methoxybenzaldehyde	8.24
3-methoxybenzoic acid	12.38
diphenylether	11.44

High performance liquid chromatography / mass spectrometry (LC–MS): Analytical LC–MS of the peptides was carried out on an *Agilent* 1100 Series Purification Platform. The HPLC system was equipped with a RP-18 column from *Macherey–Nagel* (EC-Nucleodur 250/4, 100 Å pore size, 5 μm particles) and a diode–array multi-wavelength detector. A mixture of acetonitrile and water with 0.1 % or 0.5 % of acetic acid was used as the mobile phase.

Preparative high performance liquid chromatography (preparative HPLC): The peptides were purified on a *Merck–Hitachi* NovaPrep 200 equipped with a RP-18 column from *Macherey–Nagel* (EC-Nucleodur, 100 Å pore size, 16 μm particles) and a *Merck–Hitachi* L-7400 UV detector. A mixture of acetonitrile and water with 0.1 % or 0.5 % of acetic acid was used as the mobile phase.

Ultraviolet spectroscopy (UV): The UV spectra were measured with a *Beckman Coulter* DU 800 spectrophotometer.

Microscopy: The on-bead peptide-ligands were observed with a *Zeiss* Axioplan 2, equipped with a CCD-camera. The video processing was carried out with the KS Grabbers and KS 100 software.

Scanning UV-vis spectroscopy: The screening of the library was carried out on a *Tecan* SpectraFluor Plus titerplate reader, equipped with a 310 nm absorption filter. The samples were analysed in *Nunc* 96 well titerplates and the results were processed with the Easywin software.

Laboratory journal: The number given at the beginning of each synthesis, catalysis or experimental work refers to the page in the laboratory journal.

Temperature of the reactions: All the reactions were performed at room temperature, except stated otherwise.

8.2 General protocols for solid phase peptide synthesis

8.2.1 Chemicals and special equipment

The TentaGel™ and *Wang*™ resins were purchased from *Rapp Polymere*, the tosyl chloride resin was purchased from *Argonaut Technologies* and the Fmoc-protected amino acids were purchased from *NovaBiochem*.

The peptide syntheses were performed in *Schlenk* flasks equipped with a filter plate and a gas inlet (*Merrifield* flask). The reaction mixtures were swirled on a shaker at 220 rpm.

8.2.2 Synthesis of the peptide library and encoding of the peptides

The peptide library was synthesised with the help of the *IRORI* Accu-Tag™ 100 Combinatorial Chemistry System. The synthesis was performed in MicroKan™ (capacity up to 30 mg of resin) or MacroKan™ (capacity up to 300 mg of resin) containers equipped with radiofrequency tags.

8.2.3 Swelling of the resins

Before use, the resins were allowed to swell for 1 hour. TentaGel resin was swollen in DMF, *Wang* resin was swollen in a mixture of dichloromethane and DMF (8:2) and chlorosulfonated polystyrene resin was swollen in dichloromethane.

8.2.4 Coupling with PyBOP as a coupling agent[112]

The pre-swollen resin was suspended in absolute DMF containing 3 % NMM. 2.5 eq of PyBOP and 2.5 eq of the corresponding amino acid (calculated on the loading of the resin) were added to the suspension and the reaction mixture was shaken at room temperature under argon for 12 hours. The solution turned yellow after a few hours. The solution was filtered and the resin was washed 3 times with DMF. In case of difficult sequences, the coupling was repeated 2 or 3 times.

8.2.5 Determination of free amino functions with the NF31 colour test[60]
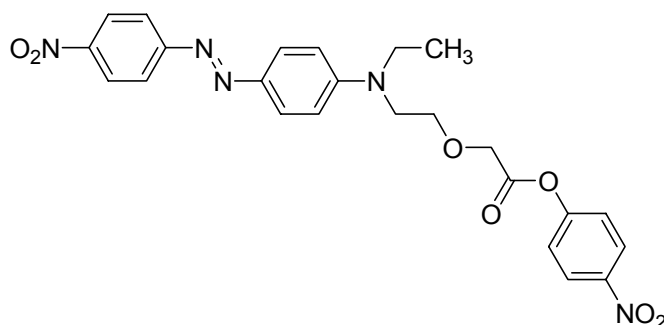
NF31, **1**

A few beads of the product were transferred into a vial and suspended in 1.00 mL of a 0.01 mM solution of **1** in acetonitrile. After heating to 70 °C for 10 minutes, the beads were washed successively 3 times with DMF, methanol and dichloromethane.

Beads containing free amino functions appeared red while completely coupled beads (without free amino functions) remained colourless.

8.2.6 Cleavage of Fmoc protecting groups and quantification of the coupling steps

8.2.6.1 Calibration curves for the determination of peptide coupling yields by UV

II-MAG-22

In order to quantify the amount of peptides attached to the resin, a calibration curve which correlated the concentration of the dibenzofulvene–piperidine adduct with the absorbance was prepared. A 100 mL graduated flask was charged with 39.0 mg (100 µmol) of Fmoc-*L*-Phe-OH and 10 mL of a 20 % piperidine solution in absolute DMF. The solution was shaken at room temperature for 10 minutes and the graduated flask was filled to 100 mL by the addition of absolute DMF. The solution was diluted with absolute DMF in order to obtain 10 samples with concentrations ranging from 0.05 to 0.50 mM. The UV absorbances at λ = 290 nm and λ = 300 nm of these samples were measured. The linear plot of the absorbance (A) against the concentration (c) of dibenzofulvene–piperidine gave the following equations (**Figure 8-1**, **Table 8-2**). These linear plots were used to determine the coupling yields during peptide synthesis.
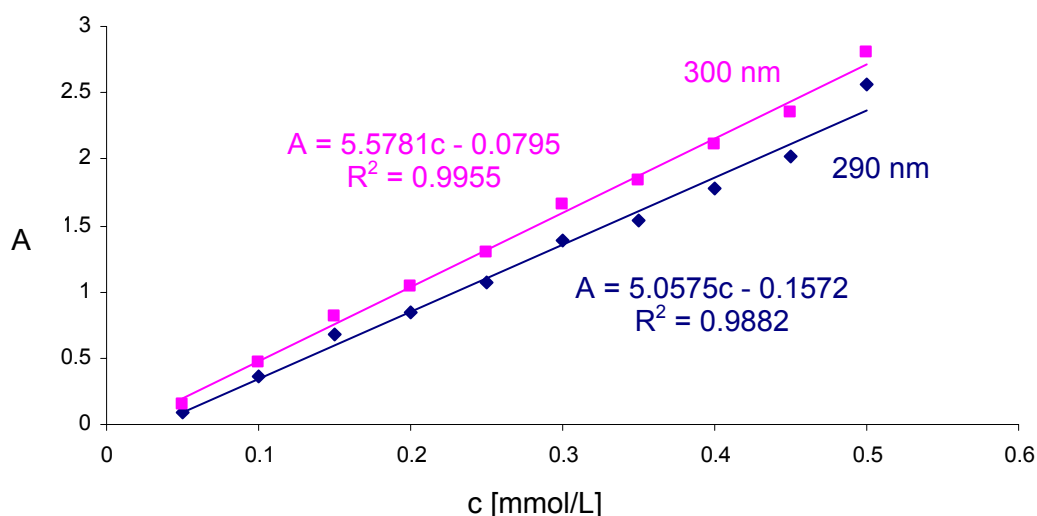
Figure 8-1: Calibration curves for the yield determination of peptide couplings per UV.

Table 8-2: **Absorbance (A) for yield determination of peptide couplings per UV.**

c [mmol/L]	A	
	λ = 290 nm	λ = 300 nm
0.05	0.0945	0.1565
0.10	0.3606	0.4731
0.15	0.6771	0.8203
0.20	0.8482	1.0474
0.25	1.0704	1.3035
0.30	1.3830	1.6603
0.35	1.5340	1.8363
0.40	1.7860	2.1031
0.45	2.0262	2.3475
0.50	2.5557	2.7966

8.2.6.2 Cleavage of Fmoc protecting groups

The resin was suspended in a 20 % solution of piperidine in absolute DMF and shaken at room temperature for 30 minutes. The treatment with piperidine was repeated twice for 20 minutes. The solution was filtered and the resin was washed 4 times with absolute DMF. The solutions were collected, combined and the UV absorbance at λ = 300 nm was measured in order to calculate the yield of the coupling.

8.2.7   Cleavage of Boc protecting groups

A 20 % solution of TFA in dry dichloromethane was added to the resin. The suspension was shaken at room temperature for 10 minutes and the resin was washed 3 times with dichloromethane to remove all traces of TFA.

8.2.8 Determination of the loading of amino TentaGel resin
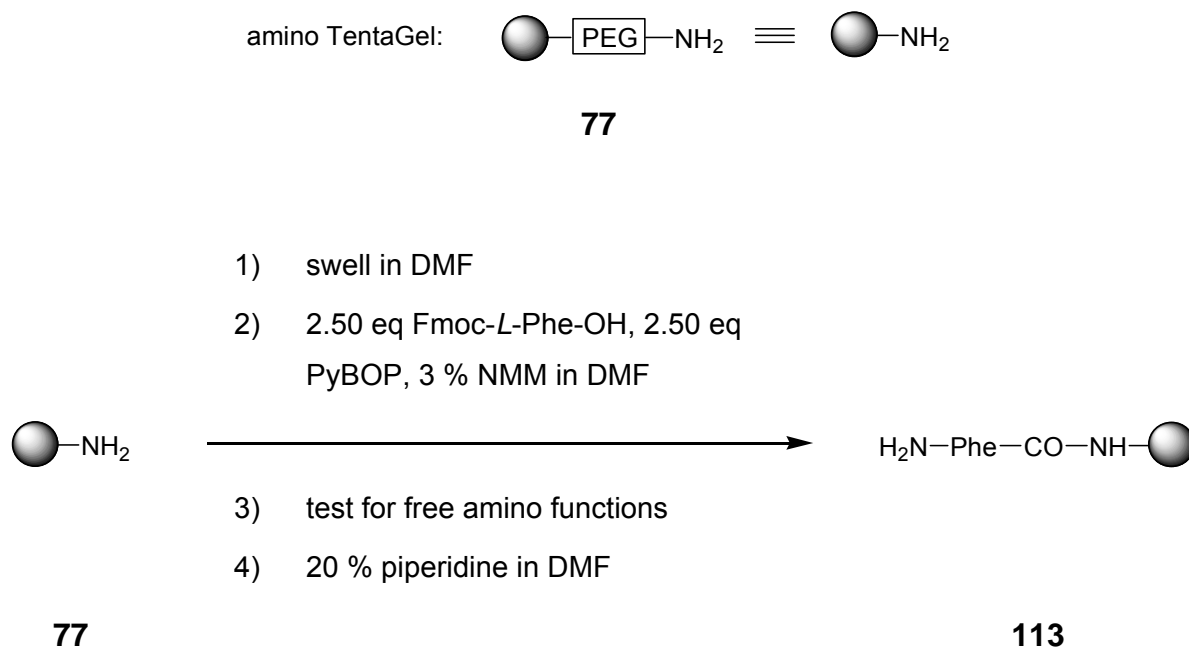
amino TentaGel: ⬤—[PEG]—NH$_2$ ≡ ⬤—NH$_2$

77

⬤—NH$_2$

1) swell in DMF

2) 2.50 eq Fmoc-*L*-Phe-OH, 2.50 eq
   PyBOP, 3 % NMM in DMF

→ H$_2$N—Phe—CO—NH—⬤

3) test for free amino functions

4) 20 % piperidine in DMF

77   **113**

A 100 mL *Merrifield* flask was charged with 100 mg (theoretical loading = 0.26 mmol/g) of amino TentaGel resin **77** and 10 mL of absolute DMF. The resin was allowed to swell by gentle shaking at room temperature for 1 hour. The solvent was filtered and 10 mL of a solution at 3 % of NMM in absolute DMF was added, as well as 25.0 mg (65.0 μmol, 2.50 eq) of Fmoc-*L*-Phe-OH and 34.0 mg (65.0 μmol, 2.50 eq) of the coupling agent PyBOP. The suspension was shaken at room temperature under argon for 12 hours. The solution was filtered and the resin was washed 3 times with 10 mL of DMF. A NF31 test was performed in order to check the completion of the reaction. Since a negative test was observed, the Fmoc protecting group was cleaved, the filtrate solutions were collected and the loading of the resin was determined with the help of the calibration curve (**Figure 8-1**). The loading of the resin was calculated to be 0.22 mmol amino functions per gram of resin.

8.2.9 Determination of the loading of *Wang* resin
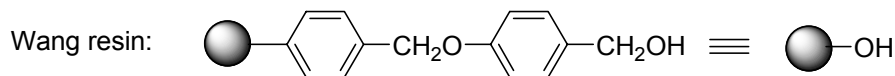
Wang resin:

91

1) swell in DMF
2) 5.00 eq Fmoc-*L*-Phe-OH, 5.00 eq DIC,
   5.00 eq HOBt, 0.10 eq DMAP
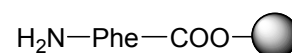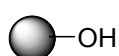
3) test for free amino functions
4) 20 % piperidine in DMF

91   **114**

A 100 mL *Merrifield* flask was charged with 100 mg (theoretical loading = 0.97 mmol/g) of *Wang* resin **91** and 10 mL of a mixture of dry dichloromethane and absolute DMF (8:2). The resin was allowed to swell by gentle shaking at room temperature for 1 hour. The solvent was filtered and a new mixture of dry dichloromethane and absolute DMF (8:2) was added, followed by 188 mg (485 μmol, 5.00 eq) of Fmoc-*L*-Phe-OH, 61.0 mg (485 μmol, 5.00 eq) of *N,N'*-di-*iso*-propyl-carbodiimide, 65.5 mg (485 μmol, 5.00 eq) of HOBt and 1.20 mg (9.70 μmol, 0.10 eq) of 4-dimethylaminopyridine. The resulting suspension was shaken at room temperature under argon for 12 hours. The solution was filtered and the resin was washed 3 times with 10 mL of dichloromethane and 3 times with 10 mL of DMF. Since an NF31 test proved the coupling to be complete, the Fmoc protecting group was cleaved with a 20 % solution of piperidine in DMF. The loading of the resin was determined to be 1.10 mmol/g.

8.3 Synthesis of the unnatural amino acid *mod*-Cys: model for the Tyr–Cys cross-link of GOase

8.3.1 Synthesis of 2,4-di-*tert*-butyl-6-bromophenol, 50[99]

I-MAG-21
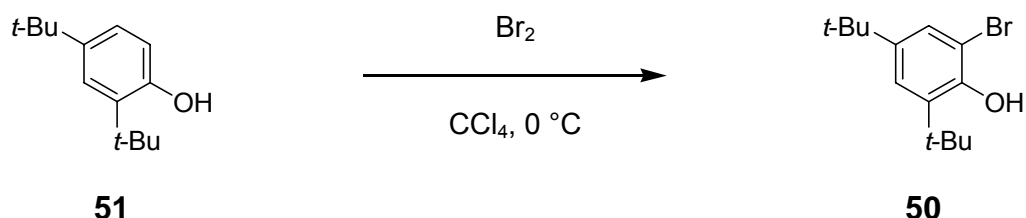
In a 250 mL three-necked flask equipped with a thermometer, a dropping funnel and a reflux condenser, a solution of 20.6 g (100 mmol, 1.00 eq) of 2,4-di-*tert*-butylphenol **51** in 100 mL of carbon tetrachloride was prepared. The solution was stirred and cooled to 0 °C. A solution of 6.00 mL (117 mmol, 1.20 eq) of bromine in 50 mL of carbon tetrachloride was added and the resulting reaction mixture was stirred at 0 °C for 3 hours. A solution of 1.00 g of sodium sulfite in 50 mL of water was added to the brown solution. The two layers were separated, the organic layer was washed with water, dried over magnesium sulphate, and the solvent was evaporated under reduced pressure to give 26.1 g (91.5 mmol, 92 %) of **50** as a pale brown solid.

50
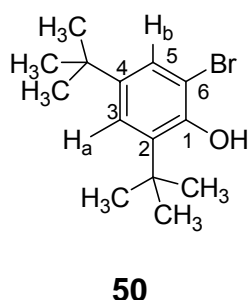
C$_{14}$H$_{21}$BrO   285.22 g·mol^{-1}

Yield   26.1 g, 91.5 mmol, 92 % (Lit.: 60 %)[99]

Melting point   56 °C (Lit.: 57–58 °C)[99]

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3512 [s, v(O–H)], 3002 [w, v(aryl–H)], 2967 [s, v(alkyl–C–H)], 2914 [m, v(alkyl–C–H)], 1574 [m, v(C=C)], 1404 [m, δ_{as}(C–H from t-Bu)], 1365 [m, δ_{s}(C–H from t-Bu)], 1281 [s, v(C–O)], 871 [s, δ_{oop}(aryl–H)], 841 [s, δ_{oop}(aryl–H)], 746 [s, δ_{oop}(aryl–H)], 715 [s, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.29 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 5.65 (s, 1H, OH), 7.25 (d, $^{4}J_{Ha,Hb}$ = 2.36, 1H, H$_a$ or H$_b$), 7.33 (d, $^{4}J_{Ha,Hb}$ = 2.36, 1H, H$_a$ or H$_b$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 30.2 (q, CH$_3$ / t-Bu), 32.3 (q, CH$_3$ / t-Bu), 35.2 (s, C$_q$ / t-Bu), 36.3 (s, C$_q$ / t-Bu), 112.7 (s, aryl–C$_6$), 124.5 (d, aryl–C$_3$), 127.0 (d, aryl–C$_5$), 137.5 (s, aryl–C$_2$ or C$_4$), 144.4 (s, aryl–C$_2$ or C$_4$), 148.8 (s, aryl–C$_1$).

TLC   R$_f$ = 0.81 (silica gel, n-hexane : dichloromethane = 2 : 1).

8.3.2 Synthesis of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene, 48[100]

I-MAG-12

- 141 -
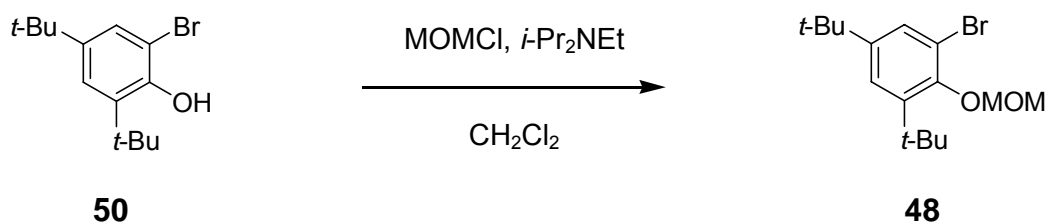
Under argon, a dry 100 mL two-necked flask was charged with 2.00 g (7.02 mmol, 1.00 eq) of 2,4-di-*tert*-butyl-6-bromophenol **50** and 20 mL of dry dichloromethane. 2.40 mL (18.9 mmol, 2.70 eq) of chloromethyl methyl ether and 3.30 mL (18.9 mmol, 2.70 eq) of di-*iso*-propylethylamine were added to the solution and the reaction mixture was stirred at room temperature under argon for 6 days. The solution turned red. 20 mL of water were added to the reaction mixture. The product was extracted with dichloromethane, the combined organic layers were washed with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. An orange oil was obtained that was purified by kugelrohr distillation ($4 \cdot 10^{-1}$ mbar, 118 °C) to give 1.90 g (5.77 mmol, 82 %) of product **48** as a colorless oil.
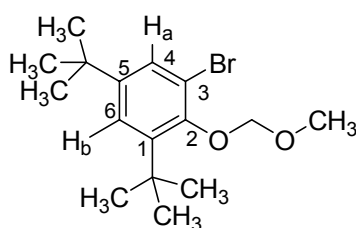
48

$C_{16}H_{25}BrO_2$ 329.27 g·mol^{-1}

Yield   1.90 g, 5.77 mmol, 82 %

FT-IR   (NaCl plate) $\tilde{\nu}$ [cm^{-1}] = 3004 [w, ν(aryl–H)], 2973 [s, ν(alkyl–C–H)], 2956 [m, ν(alkyl–C–H)], 2921 [m, ν(alkyl–C–H)], 1598 [m, ν(C=C)], 1407 [m, δ_{as}(C–H from *t*-Bu)], 1362 [m, δ_s(C–H from *t*-Bu)], 1245 [s, ν(C–O)], 1156 [s, ν(C–O)], 1079 [s, ν(C–O)], 879 [s, δ_{oop}(aryl–H)], 734 [s, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.28 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 3.68 (s, 3H, O–CH$_3$), 5.21 (s, 2H, O–CH$_2$–O), 7.30 (d, $^4J_{Ha,Hb}$ = 2.41, 1H, H$_a$ or H$_b$), 7.39 (d, $^4J_{Ha,Hb}$ = 2.41, 1H, H$_a$ or H$_b$).

¹³C-NMR (75 MHz, CDCl$_3$) δ = 30.8 (q, CH$_3$ / t-Bu), 31.3 (q, CH$_3$ / t-Bu), 34.6 (s, C$_q$ / t-Bu), 35.8 (s, C$_q$ / t-Bu), 57.7 (q, O–CH$_3$), 99.2 (t, O–CH$_2$–O), 117.4 (s, aryl–C$_3$), 123.8 (d, aryl–C$_6$), 128.7 (d, aryl–C$_4$), 142.8 (s, aryl–C$_1$ or C$_5$), 144.3 (s, aryl–C$_1$ or C$_5$), 147.5 (s, aryl–C$_2$).

TLC R$_f$ = 0.40 (silica gel, n-hexane : dichloromethane = 2 : 1).

8.3.3 Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene, 48 and reaction with the electrophile MeI[102, 103]

I-MAG-17
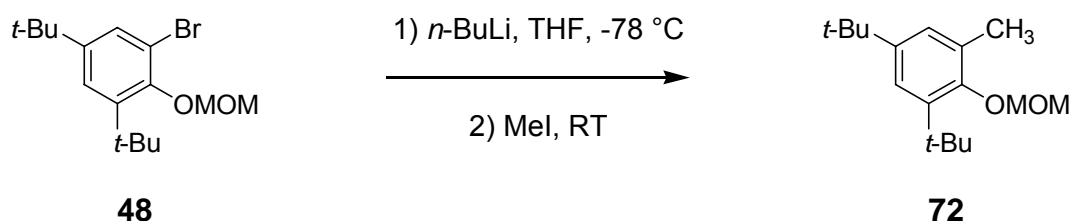
48 → **72**

Reagents: 1) n-BuLi, THF, -78 °C; 2) MeI, RT

Under argon, a dry 25 mL three-necked flask was charged with a solution of 400 mg (1.22 mmol, 1.00 eq) of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene **48** in 5 mL of dry THF. The solution was cooled down to –78 °C, 1.20 mL (1.46 mmol, 1.20 eq) of a 1.20 M n-butyllithium solution in n-hexane were added and the reaction mixture was stirred at –78 °C for one hour. 152 µl (2.44 mmol, 2.00 eq) of methyl iodide were added, the reaction mixture was allowed to warm up to room temperature and stirred for an additonal period of 36 hours. Then 5 mL of water were added to the reaction mixture. The product was extracted with ethyl acetate, washed with water, dried over magnesium sulphate, and the solvent was evaporated under reduced pressure to give 290 mg (1.10 mmol, 90 %) of **72** as a yellow oil.
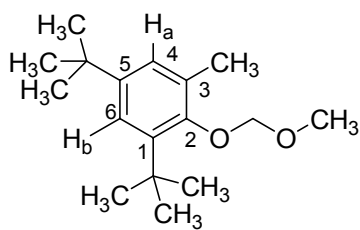
72

C$_{17}$H$_{28}$O$_2$ 264.40 g·mol^{-1}

Yield   290 mg, 1.10 mmol, 90 %

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.29 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 2.32 (s, 3H, CH$_3$), 3.65 (s, 3H, O–CH$_3$), 4.99 (s, 2H, O–CH$_2$–O), 7.04 (d, $^4J_{Ha,Hb}$ = 2.51, 1H, H$_b$), 7.19 (d, $^4J_{Ha,Hb}$ = 2.51, 1H, H$_a$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 14.6 (q, CH$_3$), 31.2 (q, CH$_3$ / *t*-Bu), 31.4 (s, C$_q$ / *t*-Bu), 31.5 (q, CH$_3$ / *t*-Bu), 40.9 (s, C$_q$ / *t*-Bu), 55.3 (q, O–CH$_3$), 95.2 (t, O–CH$_2$–O), 119.8 (d, aryl–C$_6$), 123.2 (s, aryl–C$_3$), 124.2 (d, aryl–C$_4$), 135.3 (s, aryl–C$_1$), 141.4 (s, aryl–C$_5$), 150.2 (s, aryl–C$_2$).

TLC   R$_f$ = 0.48 (silica gel, *n*-hexane : dichloromethane = 2 : 1).

8.3.4 Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene, 48 and reaction with the electrophile MeOD[102]

I-MAG-18
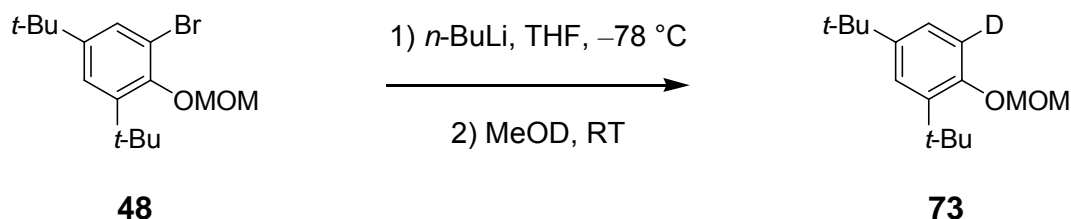
48 → **73**

1) *n*-BuLi, THF, −78 °C

2) MeOD, RT

Under argon, a dry 25 mL three-necked flask was charged with a solution of 400 mg (1.22 mmol, 1.00 eq) of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene **48** in 5 mL of dry THF. The solution was cooled down to −78 °C, 1.20 mL (1.46 mmol, 1.20 eq) of a 1.20 M *n*-butyllithium solution in *n*-hexane was added and the reaction mixture was stirred at −78 °C for one hour. 99.0 μl (2.44 mmol, 2.00 eq) of methanol-d_1 were added, the reaction mixture was allowed to warm up to room temperature and stirred for an additional period of 36 hours. Then 5 mL of water were added to the reaction mixture.The product was extracted with ethyl acetate, washed with water, dried over magnesium sulphate, and the solvent was evaporated under reduced pressure to give 305 mg (1.21 mmol, 99 %) of **73** as a white solid.
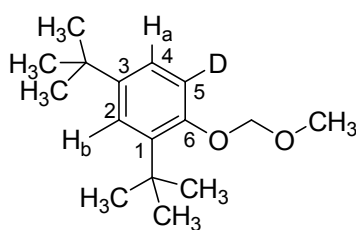
73

$C_{16}H_{25}DO_2$	251.38 g·mol^{-1}
Yield	305 mg, 1.21 mmol, 99 %

¹H-NMR   (300 MHz, CDCl₃) δ = 1.28 (s, 9H, *t*-Bu), 1.39 (s, 9H, *t*-Bu), 3.48 (s, 3H, O–CH₃), 5.19 (s, 2H, O–CH₂–O), 7.14 (td, $^4J_{Ha,Hb}$ = 2.52, $^3J_{Hb,D}$ = 3.66, 1H, Hₐ), 7.31 (d, $^4J_{Ha,Hb}$ = 2.52, 1H, H_b).

TLC R_f = 0.70 (silica gel, *n*-hexane : dichloromethane = 2 : 1).

8.3.5   Synthesis of **N,N'-bis-*tert*-butyloxycarbonyl-L-cystine-O,O'-bis-methyl ester, 49**[101]

I-MAG-25

52   **49**
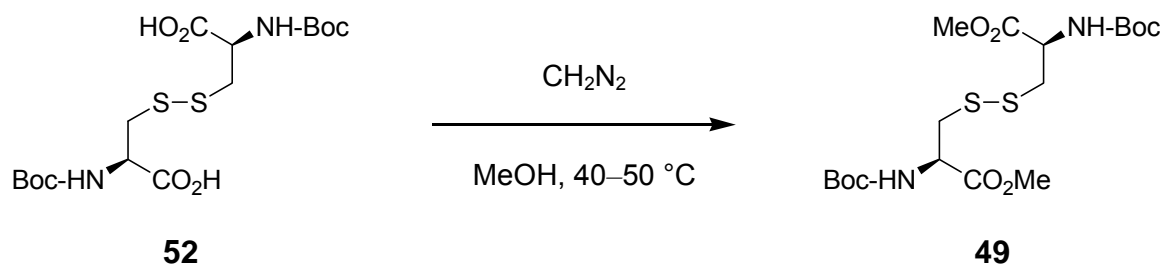
Diazomethane was formed by dropping 4.80 g (22.2 mmol, 3.00 eq) of *N*-nitroso-*N*-methyl-4-toluenesulfonamide, dissolved in 190 mL of diethylether, into a solution of 1.30 g (22.2 mmol, 3.00 eq) of potassium hydroxide in a mixture of 20 mL of ethanol and 7 mL of water. The addition was performed over 3 hours. The yellow coloured diazomethane formed was distilled at a temperature of 45 °C and came in contact with the diacid to be methylated, i.e. 3.26 g (7.39 mmol, 1.00 eq) of *N*-*N'*-bis-*tert*-butyloxycarbonyl-*L*-cystine **52** in 70 mL of methanol. Once the distillation was over, the solvent was evaporated under reduced pressure to give a colourless oil which was dissolved in a minimum of diethylether and precipitated by addition of *n*-pentane. The product was filtered to give 3.37 g (7.19 mmol, 97 %) of **49** as a white solid.
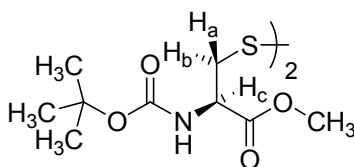
49

C$_{18}$H$_{32}$N$_2$O$_8$S$_2$ 468.59 g·mol^{-1}

Yield   3.37 g, 7.19 mmol, 97 %

Melting point   96 °C (Lit.: 96–97 °C)[122]

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 3384 [m, ν(N–H)], 2984 [w, ν(alkyl–C–H)], 1749 [s, ν(C=O from ester)], 1684 [s, ν(C=O from amide)], 1395 [m, δ$_{as}$(C–H from *t*-Bu)], 1369 [m, δ$_s$(C–H from *t*-Bu)], 1288 [m, ν(C–O)], 1255 [m, ν(C–O)], 1170 [s, ν(C–O)], 1057 [m, ν(C–O)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.43 (s, 18H, *t*-Bu), 3.15 (m, 2H, H$_a$ or H$_b$), 3.22 (m, 2H, H$_a$ or H$_b$), 3.75 (s, 6H, O–CH$_3$), 4.59 (m, 2H, H$_c$), 6.34 (s, 2H, NH).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 28.2 (q, CH$_3$ / *t*-Bu), 41.1 (t, S–CH$_2$), 52.6 (q, O–CH$_3$), 59.0 (d, CH), 80.4 (s, C$_q$ / *t*-Bu), 155.1 (s, NH–CO–O), 173.4 (s, CO–O).

TLC   R$_f$ = 0.34 (silica gel, dichloromethane).

8.3.6 Lithiation of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene, 48 and reaction with the electrophile *N,N'*-bis-*tert*-butyloxycarbonyl-*L*-cystine-*O,O'*-bis-methyl ester, 49[102]

I-MAG-20

49

48   **47**
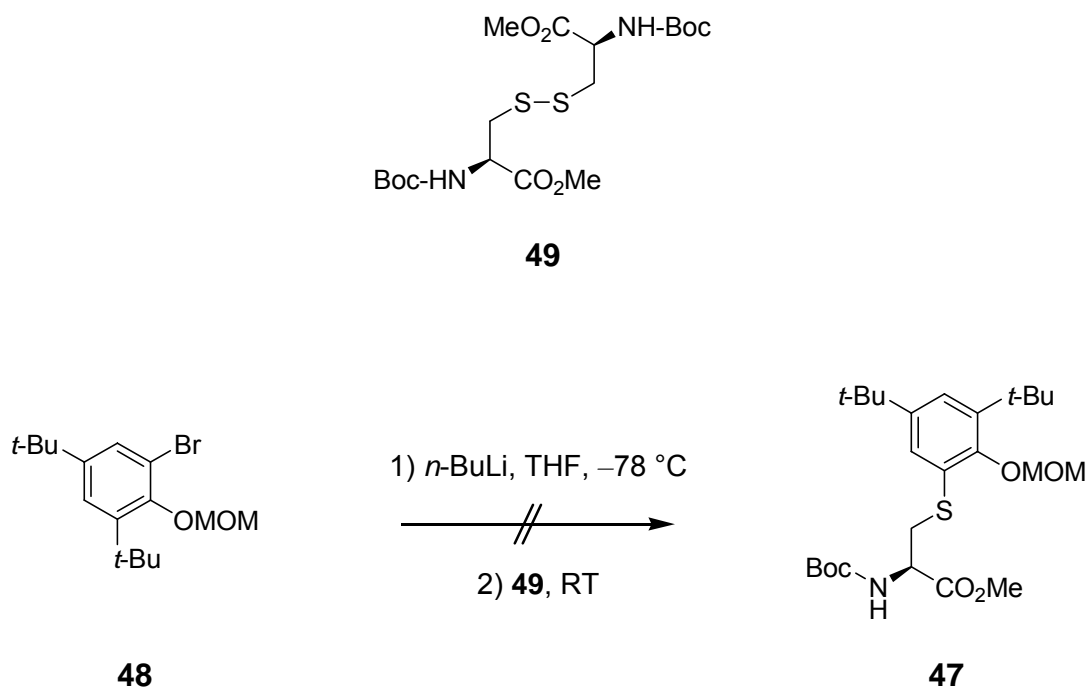
Under argon, a dry 25 mL three-necked flask was charged with a solution of 88 mg (267 µmol, 1.00 eq) of 1,5-di-*tert*-butyl-3-bromo-2-(methoxymethoxy)benzene **48** in 5 mL of dry THF. The solution was cooled down to –78 °C, 0.30 mL (320 µmol, 1.20 eq) of a 1.20 M *n*-butyllithium solution in *n*-hexane was added and the reaction mixture was stirred at –78 °C for one hour. 250 mg (535 µmol, 2.00 eq) of *N,N'*-bis-*tert*-butyloxycarbonyl-*L*-cystine-*O,O'*-bis-methyl ester **49** were added, the reaction mixture was allowed to warm up to room temperature and stirred for an additional period of 12 hours. Then 5 mL of water were added to the reaction mixture.The product was extracted with ethyl acetate, washed with water and dried over magnesium sulphate. After evaporation of the solvent under reduced pressure, the product was purified by chromatography (silica gel, *n*-hexane : dichloromethane = 2 : 1). The expected product **47** was not formed. Instead, 63 mg (252 µmol, 94 %) of the product **71** were isolated as a white solid.

71

C$_{16}$H$_{26}$O$_2$ 250.38 g·mol^{-1}

Yield   63 mg, 252 μmol, 94 %

Melting point   47 °C

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 3005 [w, ν(aryl–H)], 2961 [s, ν(alkyl–C–H)], 1605 [m, ν(C=C)], 1399 [m, δ_{as}(C–H from *t*-Bu)], 1360 [m, δ_s(C–H from *t*-Bu)], 1232 [s, ν(C–O)], 1148 [s, ν(C–O)], 1074 [s, ν(C–O)], 1005 [s, ν(C–O)], 929 [m, δ_{oop}(aryl–H)], 889 [m, δ_{oop}(aryl–H)], 820 [m, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.30 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu), 3.49 (s, 3H, O–CH$_3$), 5.20 (s, 2H, O–CH$_2$–O), 7.02 (d, $^3J_{Hb,Hc}$ = 8.56, 1H, H$_c$), 7.15 (dd, $^4J_{Ha,Hb}$ = 2.42, $^3J_{Hb,Hc}$ = 8.56, 1H, H$_b$), 7.32 (d, $^4J_{Ha,Hb}$ = 2.42, 1H, H$_a$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 29.9 (q, CH$_3$ / *t*-Bu), 31.6 (q, CH$_3$ / *t*-Bu), 34.3 (s, C$_q$ / *t*-Bu), 35.1 (s, C$_q$ / *t*-Bu), 56.0 (q, O–CH$_3$), 94.1 (t, O–CH$_2$–O), 113.6 (d, aryl–C$_6$), 123.5 (d, aryl–C$_3$ or C$_5$), 123.9 (d, aryl–C$_3$ or C$_5$), 137.4 (s, aryl–C$_2$), 143.5 (s, aryl–C$_4$), 153.9 (s, aryl–C$_1$).

TLC   R$_f$ = 0.63 (silica gel, *n*-hexane : dichloromethane = 1 : 2).

8.3.7 Synthesis of 2,4-di-*tert*-butyl-6-mercaptophenol, 64[89, 97]

I-MAG-55
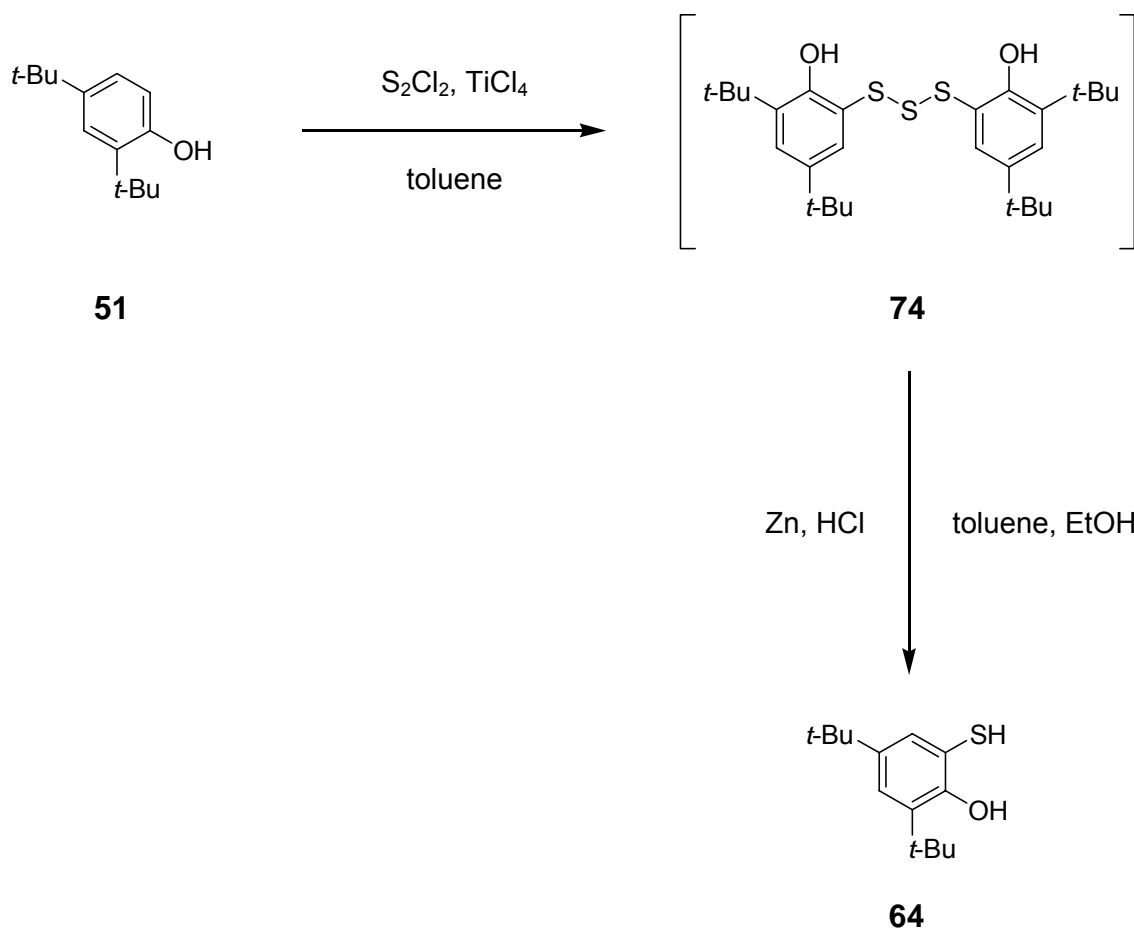
Under argon, a dry 500 mL three-necked flask was charged with a solution of 40.0 g (194 mmol, 1.00 eq) of 2,4-di-*tert*-butylphenol **51** in 200 mL of anhydrous toluene. The solution was cooled to 0 °C and 216 µL (1.94 mmol, 0.01 eq) of titanium(IV) chloride were added with a syringe. The temperature was raised up to +5 °C and 7.80 mL (97.0 mmol, 0.50 eq) of sulphur monochloride were dropped in. Once the reaction started, the temperature was lowered to –5 °C and the rest of sulphur monochloride was added. The solution was stirred at room temperature under argon for 12 hours. The reaction mixture was hydrolysed by adding 100 mL of a 5 N hydrochloric acid solution. The phases were separated and the organic phase was washed, first with a saturated solution of sodium bicarbonate, then with water. The organic layer was dried over sodium sulphate and the solvent was evaporated under

reduced pressure. The trisulfide **74** was obtained as an orange oil, which was used in the next reaction without further purification.

In a 1 L three-necked flask equipped with a condenser, the previously obtained product **74** was dissolved in 200 mL of toluene and 100 mL of ethanol. 300 mL of a 3 N hydrochloric acid solution were added. 32.0 g (485 mmol, 5.00 eq) of zinc were added in little portions to the biphasic mixture. An exothermic reaction was observed. The reaction mixture was stirred at room temperature for 12 hours. The crude product was extracted with toluene, washed with water, dried over sodium sulphate, and the solvent was evaporated under reduced pressure. The product was crystallised from 200 mL of acetonitrile and the yellow solid obtained was purified by kugelrohr distillation ($2 \cdot 10^{-1}$ mbar, 120 °C) to give 19.4 g (81.5 mmol, 42 %) of the product **64** as a pale yellow solid.
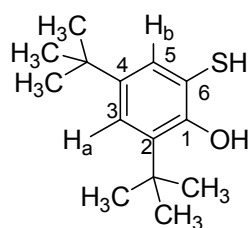
64

C$_{14}$H$_{22}$OS	238.39 g·mol^{-1}

Yield	19.4 g, 81.5 mmol, 42 % (Lit.: 23 %)[97]

Melting point	68 °C (Lit.: 70–73 °C)[97]

FT-IR	(KBr disc) $\tilde{\nu}$ [cm^{-1}] = 3414 [s, ν_s(O–H)], 2957 [s, ν_s(C–H)], 2867 [s, ν_{as}(C–H)], 2501 [m, ν_s(S–H)], 1722 [w], 1578[w], 1466 [s, δ_{as}(C–H)], 1442 [s, δ_{as}(C–H)], 1400 [m], 1361 [s, δ_s(C–H)], 1279 [s], 1246 [s], 1180 [s, ν(C–O)], 1099 [m], 924 [m], 875 [m, δ_{oop}(aryl–H)], 818 [w], 750 [w], 720 [w], 645 [w].

¹H-NMR (300 MHz, CDCl₃) δ = 1.29 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu), 2.86 (t, $^4J_{aryl-H,SH}$ = 1.02, 1H, SH), 6.62 (s, 1H, OH), 7.32 (dd, $^4J_{Ha,Hb}$ = 2.44, $^4J_{aryl-H,SH}$ = 0.94, 1H, H$_b$), 7.39 (dd, $^4J_{Ha,Hb}$ = 2.44, $J_{aryl-H,SH}$ = 1.10, 1H, H$_a$).

¹³C-NMR (75 MHz, CDCl₃) δ = 29.4 (q, CH₃ / *t*-Bu), 31.5 (q, CH₃ / *t*-Bu), 34.3 (s, C$_q$ / *t*-Bu), 35.2 (s, C$_q$ / *t*-Bu), 110.7 (s, aryl–C₆), 125.6 (d, aryl–C₃ or C₅), 130.6 (d, aryl–C₃ or C₅), 135.4 (s, aryl–C₂), 142.3 (s, aryl–C₄), 153.0 (s, aryl–C₁).

TLC R$_f$ = 0.60 (silica gel, *n*-hexane : dichloromethane = 3 : 1).

8.3.8 Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(hydroxy)phenyl]disulfane, 63[89, 97, 104]

V-MAG-2

64 → **63**
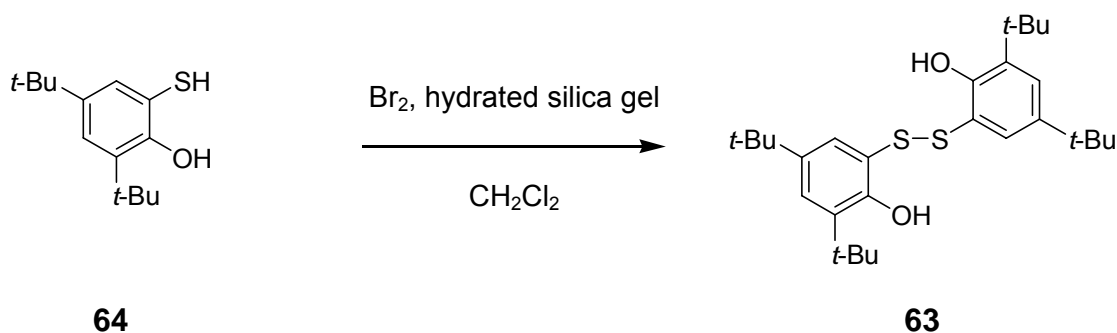
A 1 L round-bottomed flask was charged with 50.0 g of silica gel and 25 mL of water. The reaction mixture was stirred until a free flowing powder was obtained. 250 mL of dichloromethane were added, followed by a solution of 10.0 g (41.9 mmol, 1.00 eq) of 2,4-di-*tert*-butyl-6-mercaptophenol **64** in 50 mL of dichloromethane. 36.1 mL (41.9 mmol, 1.00 eq) of a 1.16 M solution of bromine in dichloromethane was added dropwise to the reaction mixture. The characteristic brown colour of the bromine

disappeared as soon as the bromine solution came in contact with the reaction mixture in the flask. When a faint brown colour persisted, the addition was stopped. The silica gel was removed by filtration, the residue was washed with dichloromethane and the solvent was evaporated under reduced pressure to give an orange oil. The product **63** was crystallised from acetonitrile to give 9.10 g (19.2 mmol, 91 %) of a yellow solid.
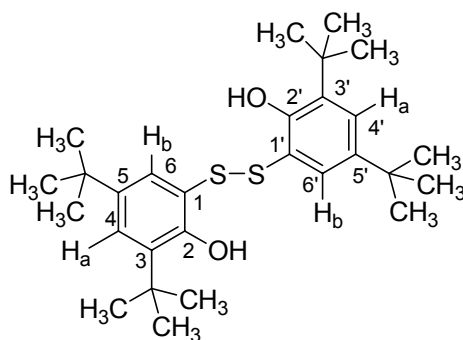
63

C$_{28}$H$_{42}$O$_2$S$_2$	474.76 g·mol^{-1}

Yield	9.10 g, 19.2 mmol, 91 %

Melting point	109 °C (acetonitrile, Lit.: 111–114 °C)[97]

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3432 [m, v(O–H)], 3430 [s, v(O–H)], 2998 [w, v(aryl–H)], 2960 [s, v(alkyl–C–H)], 2914 [m, v(alkyl–C–H)], 2868 [m, v(alkyl–C–H)], 1478 [s, δ_{as}(C–H)], 1467 [s, δ_s(C–H)], 1441 [s], 1399 [m, δ_{as}(C–H from *t*-Bu)], 1363 [s, δ_s(C–H from *t*-Bu)], 1337 [m], 1282 [m], 1245 [s], 1204 [m], 1177 [s, v(C–O)], 1137 [w], 1102 [m], 879 [w, δ_{oop}(aryl–H)], 756 [w, δ_{oop}(aryl–H)].

^{1}H-NMR (300 MHz, CDCl$_3$) δ = 1.22 (s, 18H, *t*-Bu), 1.37 (s, 18H, *t*-Bu), 6.57 (s, 2H, OH), 7.19 (d, $^4J_{Ha,Hb}$ = 2.43, 2H, H$_a$), 7.34 (d, $^4J_{Ha,Hb}$ = 2.43, 2H, H$_b$).

^{13}C-NMR (75 MHz, CDCl$_3$) δ = 29.4 (q, CH$_3$ / *t*-Bu), 31.5 (q, CH$_3$ / *t*-Bu), 34.4 (s, C$_q$ / *t*-Bu), 35.4 (s, C$_q$ / *t*-Bu), 119.4 (s, aryl–C$_1$), 128.1 (d, aryl–C$_4$ or C$_6$), 130.5 (d, aryl–C$_4$ or C$_6$), 135.7 (s, aryl–C$_3$), 142.8 (s, aryl–C$_5$), 153.3 (s, aryl–C$_2$).

MS (positive EI, MeOH) m/z (%, formula) = 474 (98, [M]$^+$), 268 (32), 238 (90, [C$_{14}$H$_{22}$OS]$^+$), 223 (95), 207 (24), 181 (64, [C$_{10}$H$_{13}$OS]$^+$), 57 (100, [C$_4$H$_9$]$^+$).

HR-MS

(positive EI)	measured	calculated [M]$^+$	$\lvert\Delta u\rvert$
	474.263	474.2626	0.005

Elemental analysis

	C	H
calculated [%]	70.84	8.92
measured [%]	70.98	9.02
accuracy	0.20	1.12

TLC R$_f$ = 0.62 (silica gel, *n*-hexane : dichloromethane = 6 : 1).

X-ray

temperature	293(2) K
wavelength	0.71073 Å
crystal system, space group	triclinic, *P*$^{-1}$
unit cell dimensions	a = 9.9511(10) Å, α = 73.356(6)°
	b = 10.1570(12) Å, β = 83.200(6)°
	c = 14.8759(9) Å, γ = 76.789(5)°

volume	1400.2(2) Å^3
Z	2
calculated density	1.126 g/cm^3
absorption coefficient	0.211 mm^{-1}
F(000)	516
crystal size	0.2 x 0.2 x 0.3 mm
Θ range	1.43° until 27.42°
limiting indices	$-12 < h < 7$
	$-13 < k < 12$
	$-19 < l < 14$
reflections collected / unique	7846 / 6003 [R(int) = 0.0325]
reflection observed [I>2σ(I)]	3780
refinement method	full-matrix least squares on F^2
data / restraints / parameters	6003 / 6 / 451
goodness-of-fit on F^2	0.983
R indices [I>2σ(I)]	R1 = 0.0526, wR2 = 0.1286
R indices (all data)	R1 = 0.0982, wR2 = 0.1497
largest diff. peak and hole	0.324 and -0.446 eÅ^{-3}

8.3.9 Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(allyloxy)phenyl]disulfane, 62[105]

II-MAG-61
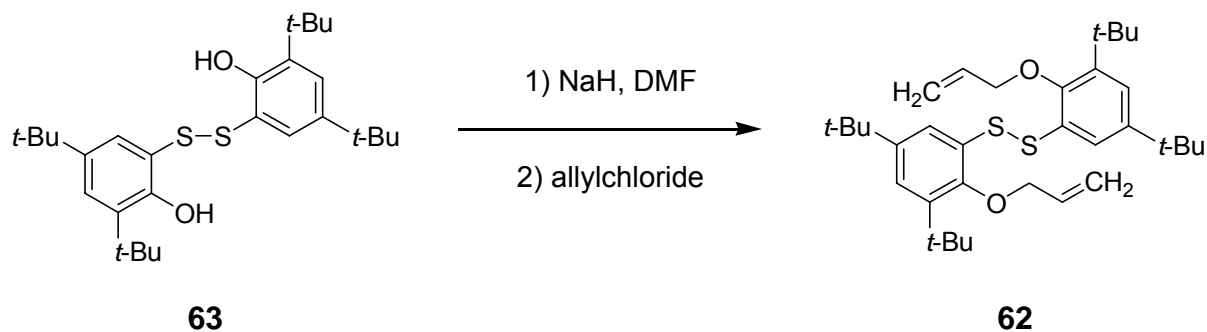
63 → **62**

Under argon, a dry 100 mL three-necked flask equipped with a condenser was charged with a solution of 1.00 g (2.11 mmol, 1.00 eq) of 1,1'-bis(3,5-di-*tert*-butyl-2-(hydroxy)phenyl)disulfane **63** in 25 mL of absolute DMF. To this solution 510 mg (21.1 mmol, 10.0 eq) of sodium hydride were added. The solution turned red rapidly. It was stirred at room temperature for an hour. Then 0.70 mL (8.44 mmol, 4.00 eq) of allylchloride was added and the reaction mixture was stirred for an additional period of 12 hours at room temperature. The reaction mixture was hydrolysed by adding 20 mL of water, the product was extracted with dichloromethane, washed with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The product was crystallised from acetonitrile to give 770 mg (1.39 mmol, 66 %) of **62** as a white solid.

62

C$_{34}$H$_{50}$O$_2$S$_2$ 554.89 g·mol^{-1}

Yield   770 mg, 1.39 mmol, 66 %

Melting point   112 °C (acetonitrile)

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 2962 [s, ν(aryl–H)], 2921 [m, ν(alkyl–C–H)], 2869 [m, ν(alkyl–C–H)], 1619 [w, ν(C=C)], 1590 [w, ν(C=C)], 1566 [w, ν(C=C)], 1471 [s, δ_{as}(C–H)], 1436 [s, δ_s(C–H)], 1398 [m, δ_{as}(C–H from *t*-Bu)], 1362 [s, δ_s(C–H from *t*-Bu)], 1283 [m], 1228 [s], 1102 [m, ν(C–O)], 980 [s, δ_{oop}(C=CH$_2$)], 931 [m, δ_{oop}(C=CH$_2$)], 880 [w, δ_{oop}(aryl–H)], 823 [w, δ_{oop}(aryl–H)], 763 [w, δ_{oop}(aryl–H)], 648 [w, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.22 (s, 18H, *t*-Bu), 1.41 (s, 18H, *t*-Bu), 4.58–4.60 (d, ^3J = 5.14, 4H, O–CH$_2$), 5.29–5.33 (dd, ^3J = 10.5, ^2J = 1.49, 2H, =CH$_{2\text{-trans}}$), 5.48–5.54 (dd, ^3J = 17.2, ^2J = 1.68, 2H, =CH$_{2\text{-cys}}$), 6.09–6.23 (m, 2H, HC=), 7.20 (d, $^4J_{Ha,Hb}$ = 2.38, 2H, H$_a$), 7.48 (d, $^4J_{Ha,Hb}$ = 2.38, 2H, H$_b$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 29.0 (q, CH$_3$ / *t*-Bu), 30.9 (q, CH$_3$ / *t*-Bu), 34.3 (s, C$_q$ / *t*-Bu), 35.1 (s, C$_q$ / *t*-Bu), 73.9 (t, O–CH$_2$), 116.6 (t, =CH$_2$), 123.2 (d, aryl–C$_4$ or C$_6$), 124.3 (d, aryl–C$_4$ or C$_6$), 129.7 (s, aryl–C$_1$), 133.2 (d, HC=), 142.1 (s, aryl–C$_3$), 146.0 (s, aryl–C$_5$), 152.8 (s, aryl–C$_2$).

MS   (positive EI, MeOH) m/z (%, formula) = 554 (12, [M]$^+$), 277 (100, [C$_{17}$H$_{25}$OS]$^+$), 57 (78, [C$_4$H$_9$]$^+$).

HR-MS	(positive EI)	measured	calculated [M]$^+$	$\|\Delta u\|$
		554.325	554.3252	0.005

Elemental analysis		C	H
	calculated [%]	73.59	9.08
	measured [%]	73.52	9.18
	accuracy	0.10	1.10

TLC $R_f = 0.65$ (silica gel, *c*-hexane : dichloromethane = 6 : 1).

8.3.10 Synthesis of 3,5-di-*tert*-butyl-2-(allyloxy)benzenethiol, 56[106]

II-MAG-75

62 **56**

56 **75**

56   **62**
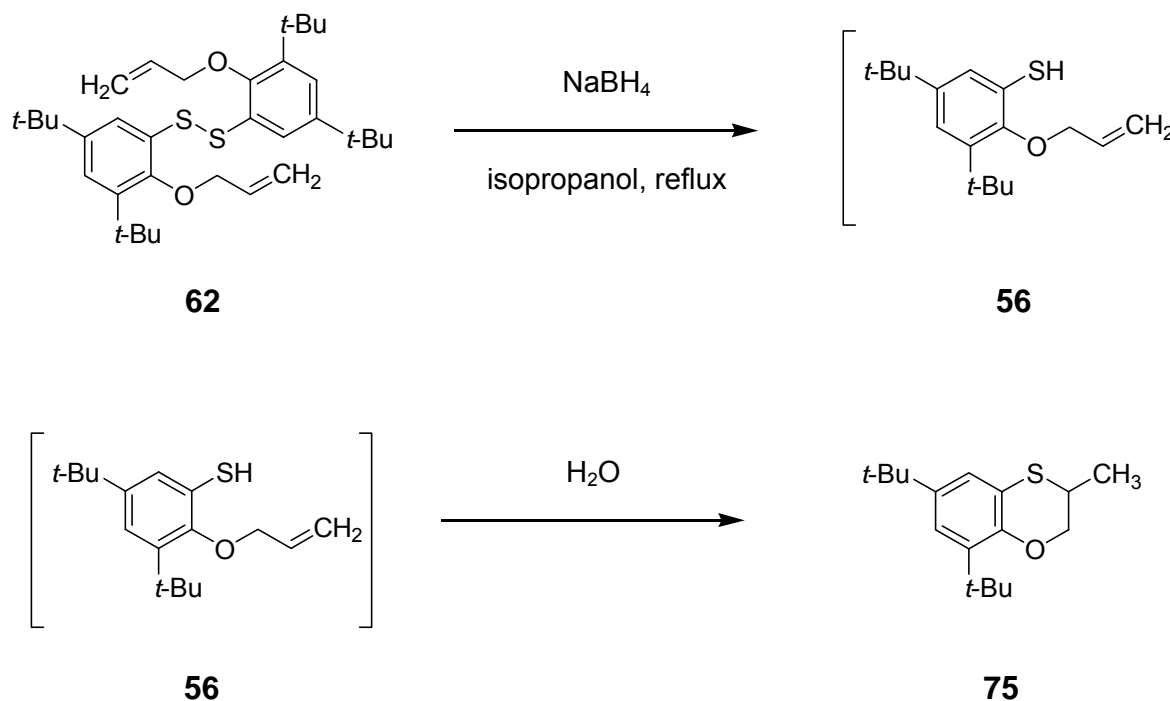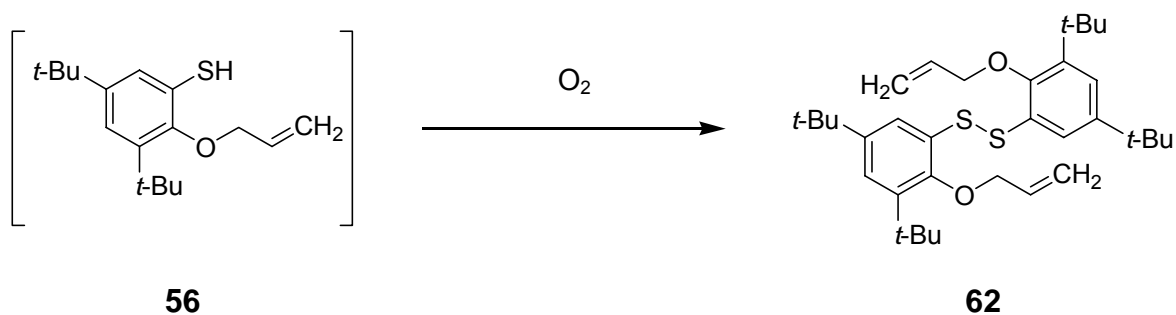
Under argon, a dry 50 mL three-necked flask equipped with a condenser was charged with a solution of 100 mg (200 μmol, 1.00 eq) of 1,1'-bis-(3,5-di-*tert*-butyl-2-(allyloxy)phenyl)disulfane **62** in 15 mL of anhydrous isopropanol. To this solution 35.0 mg (800 μmol, 4.00 eq) of sodium borohydride were added and the reaction mixture was stirred under reflux of isopropanol for 30 minutes. The solution was hydrolysed by adding 10 mL of water, the product was extracted with dichloromethane, washed with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The product was analysed by NMR directly after the purification and product **56** could be identified, but it cyclised rapidly to form the product **75**. 110 mg (390 μmol, 99 %) of product **75** were obtained as a yellow solid. Stored under dioxygen, product **56** dimerises to the disulfide **62**. In a similar experiment where **56** was stored under air, 95.0 mg (170 μmol, 85 %) of the starting material **62** were isolated.
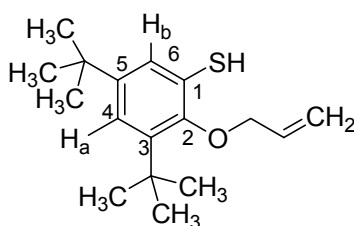
56

C$_{17}$H$_{26}$OS   278.45 g·mol^{-1}

Conversion   quant. (verified by TLC and NMR)

¹H-NMR   (300 MHz, CDCl$_3$) δ = 1.29 (s, 18H, *t*-Bu), 1.40 (s, 18H, *t*-Bu), 3.61 (s, 1H, SH), 4.55 (d, ^3J = 4.68, 2H, O–CH$_2$), 5.32 (dd, ^3J = 10.3, ^2J = 1.00, 1H, =CH$_{2\text{-trans}}$), 5.53 (dd, ^3J = 17.0, ^2J = 1.32, 1H, =CH$_{2\text{-cys}}$), 6.07–6.20 (m, 1H, HC=), 7.16 (s, 2H, H$_a$+H$_b$).

¹³C-NMR   (75 MHz, CDCl$_3$) δ = 30.9 (s, C$_q$ / *t*-Bu), 31.0 (q, CH$_3$ / *t*-Bu), 31.4 (q, CH$_3$ / *t*-Bu), 31.5 (s, C$_q$ / *t*-Bu), 71.6 (t, O–CH$_2$), 116.6 (t, =CH$_2$), 122.4 (s, aryl–C$_1$), 125.5 (d, HC=), 133.7 (d, aryl–C$_4$ or C$_6$), 134.4 (d, aryl–C$_4$ or C$_6$), 142.9 (s, aryl–C$_3$), 146.4 (s, aryl–C$_5$), 151.9 (s, aryl–C$_2$).

TLC   R$_f$ = 0.68 (silica gel, *c*-hexane).
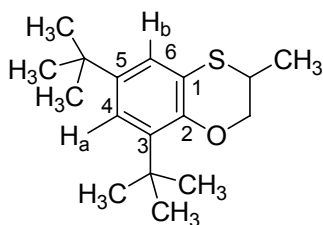
75

C$_{17}$H$_{26}$OS   278.45 g·mol^{-1}

Yield   110 mg, 390 μmol, 99 %

Melting point   109 °C (acetonitrile)

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 2961 [s, ν(aryl–H)], 2920 [m, ν(alkyl–C–H)], 2876 [m, ν(alkyl–C–H)], 1607 [m, ν(C=C)], 1570 [w, ν(C=C)], 1566 [w, ν(C=C)], 1473 [s, δ_{as}(C–H)], 1436 [s, δ_s(C–H)], 1262 [s, ν_{as}(C–O)], 1224 [m], 1096 [s], 1036 [s, ν_s(C–O)], 862 [w, δ_{oop}(aryl–H)], 812 [s, δ_{oop}(aryl–H)], 800 [s, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.26–1.36 (d, ^3J = 29.1, 3H, CH$_3$), 1.41 (s, 18H, 2 x t-Bu), 3.45–3.56 (m, 1H, CH), 3.85 (dd, ^2J = 11.1, ^3J = 7.86, 1H, CH$_2$), 4.35–4.39 (dd, ^2J = 11.1, ^3J = 2.97, 1H, CH$_2$), 6.89 (d, $^4J_{Ha,Hb}$ = 2.38, 2H, H$_b$), 7.02 (d, $^4J_{Ha,Hb}$ = 2.38, 2H, H$_a$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 29.9 (q, CH$_3$ / t-Bu), 31.1 (q, CH$_3$), 31.6 (q, CH$_3$ / t-Bu), 34.3 (d, CH), 34.5 (s, C$_q$ / t-Bu), 35.2 (s, C$_q$ / t-Bu), 70.4 (t, O–CH$_2$), 118.5 (s, aryl–C$_1$), 120.4 (d, aryl–C$_6$), 121.7 (d, aryl–C$_4$), 138.7 (s, aryl–C$_3$), 143.7 (s, aryl–C$_5$), 148.5 (s, aryl–C$_2$).

MS   (positive EI, CH$_2$Cl$_2$) m/z (%, formula) = 278 (50, [M]$^+$), 263 (100, [M – CH$_3$]$^+$), 57 (34, [C$_4$H$_9$]$^+$).

HR-MS   (positive EI)   measured   calculated [M]$^+$ |Δu|
                              278.171   278.1704   0.005

TLC   R$_f$ = 0.47 (silica gel, c-hexane).

8.3.11 Synthesis of 1,1'-bis-[3,5-di-*tert*-butyl-2-(methoxymethoxy)phenyl]-disulfane, 61[100]

II-MAG-14

63   **61**
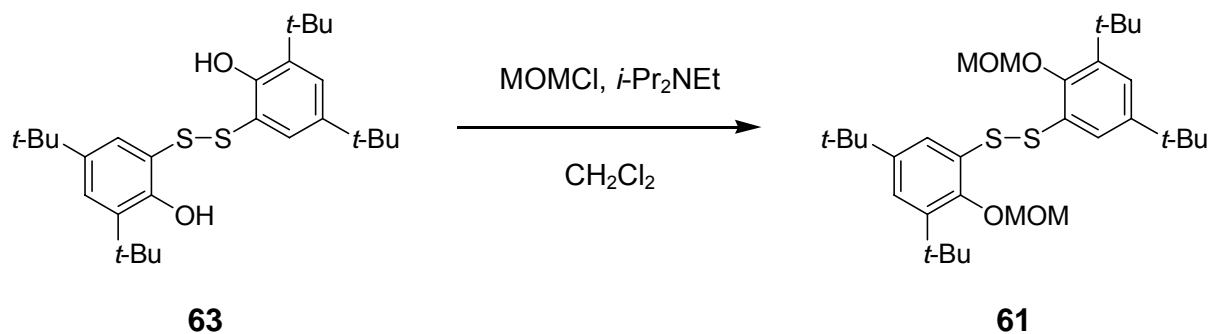
In a 250 mL dry three-necked flask under argon, 18.7 g (39.0 mmol, 1.00 eq) of 1,1'-bis-(3,5-di-*tert*-butyl-2-(hydroxy)phenyl)disulfane **63** were dissolved in 110 mL of dry dichloromethane. To this solution 37.0 mL (213 mmol, 5.40 eq) of di-*iso*-propylethylamine and 28.0 mL (213 mmo l, 5.40 eq) of chloromethyl methyl ether were added. The reaction mixture was stirred at room temperature under argon for 12 hours. The reaction mixture was hydrolysed by adding 100 mL of water, the product was extracted with dichloromethane, washed with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. Finally the product was purified by flash chromatography (silica gel, *n*-hexane : dichloromethane = 1 : 2) to yield 19.0 g (33.8 mmol, 87 %) of product **61** as a white solid. The product was recrystallised from acetonitrile.

61

C$_{32}$H$_{50}$O$_4$S$_2$	562.87 g·mol^{-1}

Yield   19.0 g, 33.8 mmol, 87 %

Melting point   41 °C (acetonitrile)

FT-IR   (KBr disc) $\tilde{\nu}$ [cm^{-1}] = 2953 [s, ν(aryl–H)], 2871 [m, ν(alkyl–C–H)], 2856 [m, ν(alkyl–C–H)], 1651 [w, ν(C=C)], 1558 [w, ν(C=C)], 1450 [m, δ_{as}(C–H)], 1434 [s, δ_s(C–H)], 1396 [m, δ_{as}(C–H from *t*-Bu)], 1361 [s, δ_s(C–H from *t*-Bu)], 1282 [m], 1234 [m], 1200 [s, ν(C–O)], 1169 [s, ν(C–O)], 1083 [s, ν(C–O)], 929 [s], 907 [s], 881 [w, δ_{oop}(aryl–H)], 764 [w, δ_{oop}(aryl–H)], 648 [w, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.20 (s, 18H, *t*-Bu), 1.41 (s, 18H, *t*-Bu), 3.67 (s, 6H, O–CH$_3$), 5.09 (s, 4H, O–CH$_2$–O), 7.23 (d, $^4J_{Ha,Hb}$ = 2.45, 2H, H$_a$), 7.46 (d, $^4J_{Ha,Hb}$ = 2.45, 2H, H$_b$).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 31.0 (q, CH$_3$ / *t*-Bu), 31.3 (q, CH$_3$ / *t*-Bu), 31.3 (s, C$_q$ / *t*-Bu), 35.5 (s, C$_q$ / *t*-Bu), 57.7 (q, O–CH$_3$), 99.8 (t, O–CH$_2$–O), 124.3 (d, aryl–C$_4$ or C$_6$), 125.8 (d, aryl–C$_4$ or C$_6$), 130.0 (s, aryl–C$_1$), 142.8 (s, aryl–C$_3$), 146.7 (s, aryl–C$_5$), 151.6 (s, aryl–C$_2$).

MS   (positive EI, CH$_2$Cl$_2$) m/z (%, formula) = 562 (15, [M]$^+$), 281 (50, [C$_{16}$H$_{25}$O$_2$S]$^+$), 250 (100, [C$_{16}$H$_{25}$O$_2$S – 2 × CH$_3$ – H]$^+$), 235 (45, [C$_{16}$H$_{25}$O$_2$S – 3 × CH$_3$ – H]$^+$), 193 (40), 57 (18, [C$_4$H$_9$]$^+$).

HR-MS

(positive EI)	measured	calculated [M]$^+$	|Δu|
	562.315	562.3150	0.005

Elemental analysis		C	H
	calculated [%]	68.28	8.95
	measured [%]	68.26	9.08
	accuracy	0.03	1.45

TLC R_f = 0.66 (silica gel, *n*-hexane : dichloromethane = 1 : 2).

8.3.12 Synthesis of 3,5-di-*tert*-butyl-2-(methoxymethoxy)benzenethiol, 55[106]

II-MAG-16

61   **55**
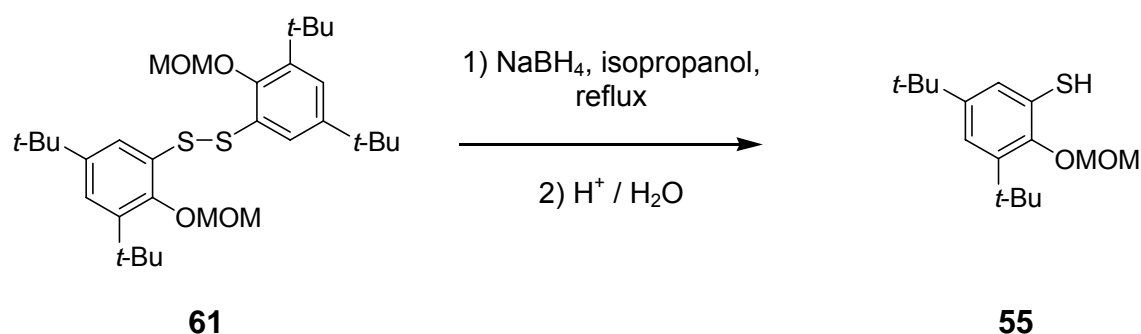
Under argon, in a dry 500 mL three-necked flask equipped with a condenser, 19.0 g (34.0 mmol, 1.00 eq) of 1,2-bis-(3,5-di-*tert*-butyl-2-(methoxymethoxy)phenyl)disulfane **61** were dissolved in 220 mL of dry isopropanol. To this solution 3.80 g (101 mmol, 3.00 eq) of sodium borohydride were added and the reaction mixture was stirred under reflux of isopropanol for one hour. The reaction mixture was cooled down to room temperature and the solution was hydrolysed with 100 mL of a 0.05 N hydrochloric acid solution. The product was extracted with dichloromethane, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The crude product was immediately purified by chromatography (aluminium oxide, *c*-hexane : dichloromethane = 1 : 2) to give 17.0 g (60.0 mmol, 89 %) of product **55** as a white solid. The purified product **55** was stable in the fridge for several months without decomposition.
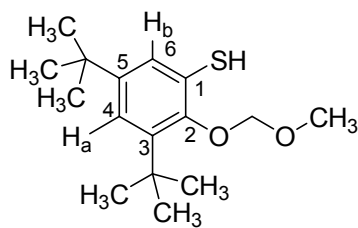
55

C$_{16}$H$_{26}$O$_2$S   282.44 g·mol^{-1}

Yield   17.0 g, 60.0 mmol, 89 %

Melting point   32 °C (acetonitrile)

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 2959 [s, ν(aryl–H)], 2873 [m, ν(alkyl–C–H)], 2855 [m, ν(alkyl–C–H)], 2569 [w, ν(S–H)], 1590 [w, ν(C=C)], 1559 [w, ν(C=C)], 1472 [m, δ_{as}(C–H)], 1436 [m, δ_s(C–H)], 1396 [s, δ_{as}(C–H from *t*-Bu)], 1362 [s, δ_s(C–H from *t*-Bu)], 1284 [m], 1231 [m], 1199 [s, ν(C–O)], 1165 [s, ν(C–O)], 1075 [s, ν(C–O)], 959 [s], 951 [s], 852 [w, δ_{oop}(aryl–H)], 749 [w, δ_{oop}(aryl–H)], 614 [w, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.27 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu), 3.67 (s, 3H, O–CH$_3$), 3.85 (s, 1H, SH), 5.12 (s, 2H, O–CH$_2$–O), 7.15 (s, 2H, aryl).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 31.1 (q, CH$_3$ / *t*-Bu), 31.3 (q, CH$_3$ / *t*-Bu), 34.5 (s, C$_q$ / *t*-Bu), 35.5 (s, C$_q$ / *t*-Bu), 57.5 (q, O–CH$_3$), 98.3 (t, O–CH$_2$–O), 122.3 (d, aryl–C$_4$), 125.4 (s, aryl–C$_1$), 125.7 (d, aryl–C$_6$), 143.2 (s, aryl–C$_3$), 146.6 (s, aryl–C$_5$), 149.9 (s, aryl–C$_2$).

MS (positive EI, CH_2Cl_2) m/z (%, formula) = 282 (12, $[M]^+$), 250 (38, [M − S]$^+$), 235 (100, $[M - S - CH_3]^+$), 57 (18, $[C_4H_9]^+$).

HR-MS (positive EI)

	measured	calculated $[M]^+$	$\lvert \Delta u \rvert$
	282.164	282.1653	0.005

TLC R_f = 0.72 (silica gel, *n*-hexane : dichloromethane = 1 : 2).

X-ray

temperature	100(2) K
wavelength	0.71073 Å
crystal system, space group	monoclinic, *C* 2/c
unit cell dimensions	a = 38.660(4) Å, α = 90°
	b = 5.8546(4) Å, β = 122.965(2)°
	c = 34.053(4) Å, γ = 90°
volume	6466.5(11) Å^3
Z	16
calculated density	1.160 g/cm^3
absorption coefficient	0.197 mm^{-1}
F(000)	2464
crystal size	0.2 x 0.3 x 0.3 mm
Θ range	2.11° until 27.00°
limiting indices	−48 < h < 30
	−7 < k < 5
	−36 < l < 43
reflections collected / unique	12646 / 6669 [R(int) = 0.0576]
reflection observed [I>2σ(I)]	3447
refinement method	full-matrix least squares on F^2

data / restraints / parameters	6669 / 0 / 397
goodness-of-fit on F^2	0.934
R indices [I>2σ(I)]	R1 = 0.0559, wR2 = 0.1243
R indices (all data)	R1 = 0.1356, wR2 = 0.1479
largest diff. peak and hole	0.688 and −0.293 eÅ^{-3}

8.3.13 Synthesis of *N*-(*tert*-butyloxycarbonyl)-*L*-serine-β-lactone, 57[89, 95, 107]

II-MAG-30
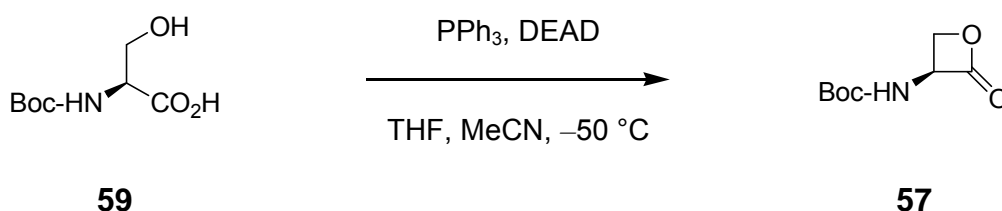
59   **57**

Under argon, a dry 250 mL three-necked flask equipped with a dropping funnel was charged with 6.70 g (25.6 mmol, 1.05 eq) of dry triphenylphosphine and a mixture of 90 mL of dry acetonitrile and 10 mL of dry THF. The solution was cooled down to -50 °C, 4.00 mL (25.6 mmol, 1.05 eq) of diethylazodicarboxylate were added and the reaction mixture was stirred at −50 °C under argon for one hour. Then a solution of 5.00 g (24.4 mmol, 1.00 eq) of *N*-(*tert*-butyloxycarbonyl)-*L*-serine **59** in a mixture of 90 mL of dry acetonitrile and 10 mL of dry THF was added slowly. The reaction mixture was stirred at −50 °C for 3 hours, then at room temperature overnight. The solvent was evaporated under reduced pressure and the product was purified by chromatography (silica gel, *c*-hexane : ethyl acetate = 6.5 : 3.5) to give 2.50 g (13.4 mmol, 55 %) of product **57** as a white solid.

57

C$_8$H$_{13}$NO$_4$	187.19 g·mol^{-1}

Yield　　　　　2.50 g, 13.4 mmol, 55 % (Lit.: 72 %)[95]

Melting point　119 °C (Lit.: 120 °C)[95]

FT-IR　　　　(CsI disc) $\tilde{v}$ [cm^{-1}] = 3363 [s, ν(N–H)], 2979 [m, ν(alkyl–C–H)], 1845 [s, ν(O–C=O)], 1682 [s, ν(N–C=O)], 1532 [s, δ(C–H)], 1373 [s, δ(C–H from *t*-Bu)], 1292 [s], 1261 [s], 1166 [s, ν(C–O)], 1106 [s, ν(C–O)], 1010 [m], 891 [s], 624 [m].

^{1}H-NMR　　(300 MHz, CDCl$_3$) δ = 1.44 (s, 9H, *t*-Bu), 4.37–4.44 (m, 2H, CH$_2$), 5.05–5.09 (m, 1H, CH), 5.27 (s, br, 1H, NH).

^{13}C-NMR　(75 MHz, CDCl$_3$) δ = 28.3 (q, CH$_3$ / *t*-Bu), 59.6 (d, CH), 66.8 (t, CH$_2$), 81.6 (s, C$_q$ / *t*-Bu), 154.6 (s, N–C=O), 169.3 (s, O–C=O).

Specific rotation

[α]$^{22}_{589}$ = –4.3° (CHCl$_3$, c = 1.00)

[α]$^{22}_{405}$ = –14.3° (CHCl$_3$, c = 1.00)

[α]$^{22}_{589}$ = –9.1° (CH$_2$Cl$_2$, c = 0.93) (Lit.: –10.8 °)[123]

TLC　　　　　R$_f$ = 0.38 (silica gel, *c*-hexane : ethyl acetate = 6.5 : 3.5, visualised with phosphomolybdic acid).

8.3.14 Synthesis of *N*-(benzyloxycarbonyl)-*L*-serine-β-lactone, 58[95]

IV-MAG-60
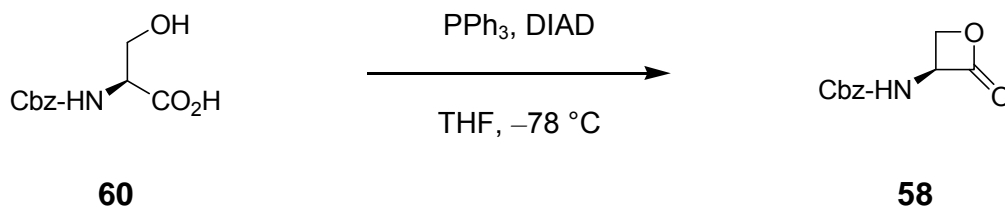
Under argon, a dry 250 mL three-necked flask equipped with a dropping funnel was charged with 6.70 g (25.5 mmol, 1.05 eq) of dry triphenylphosphine and 100 mL of dry THF. The solution was cooled down to −78 °C, 5.10 mL (25.5 mmol, 1.05 eq) of di-*iso*-propylazodicarboxylate were added and the reaction mixture was stirred at -78 °C under argon for 30 minutes. A solution of 5.80 g (24.2 mmol, 1.00 eq) of *N*-(benzyloxycarbonyl)-*L*-serine **60** in 100 mL of dry THF was added slowly. The reaction mixture was stirred at −78 °C for 3 hours and at room temperature overnight. The solvent was evaporated under reduced pressure and the product was isolated by 2 successive chromatographic columns (silica gel, *c*-hexane : ethyl acetate = 11 : 9 and dichloromethane : ethyl acetate = 18 : 2) to give 2.60 g (11.8 mmol, 49 %) of product **58** as a white solid.
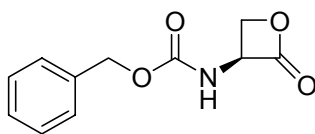
58

C₁₁H₁₁NO₄ 221.21 g·mol⁻¹

Yield 2.60 g, 11.8 mmol, 49 % (Lit.: 60 %)[95]

Melting point 129-130 °C (Lit.: 133–134 °C)[95]

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3367 [s, ν(N–H)], 2996 [m, ν(aryl–H)], 2983 [w, ν(alkyl–C–H)], 1848 [s, ν(O–C=O)], 1688 [s, ν(N–C=O)], 1606 [s, ν(C=C)], 1533 [s, ν(C=C)], 1466 [m, δ(C–H)], 1378 [m, δ_{as}(C–H from *t*-Bu)], 1346 [m, δ_s(C–H from *t*-Bu)], 1271 [s], 1195 [w], 1110 [s, ν(C–O)], 1020 [s, ν(C–O)], 885 [s, δ_{oop}(aryl–H)], 754 [s, δ_{oop}(aryl–H from benzyl)], 703 [m, δ_{oop}(aryl–H from benzyl)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 4.43–4.45 (m, 2H, CH$_2$–(CH)), 5.14 (m, 3H, CH$_2$–Ph + CH–(NH)), 5.56 (s, br, 1H, NH), 7.35 (s, 5H, aryl–H).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 59.6 (d, CH–(NH)), 66.3 (t, CH$_2$–Ph), 67.8 (t, CH$_2$), 128.3 (d, aryl–CH), 128.5 (d, aryl–CH), 128.6 (d, aryl–CH), 135.4 (s, aryl–C), 155.2 (s, N–C=O), 168.7 (s, O–C=O).

Specific rotation $[\alpha]_{589}$ = –4.4° (CHCl$_3$, c = 1.00)

$[\alpha]_{546}$ = –5.1° (CHCl$_3$, c = 1.00)

$[\alpha]_{405}$ = –14.6° (CHCl$_3$, c = 1.00)

$[\alpha]_{365}$ = –21.0° (CHCl$_3$, c = 1.00)

$[\alpha]_{334}$ = –28.9° (CHCl$_3$, c = 1.00)

MS   (dip/ms) m/z (%, formula) = 221 (10, [M]$^+$), 177 (38), 91 (100, [Ph-CH$_2$]$^+$).

TLC   R$_f$ = 0.59 (silica gel, dichloromethane : ethyl acetate = 18 : 2, visualized with phosphomolybdic acid).

8.3.15 Synthesis of *N*-(*tert*-butyloxycarbonyl)-*S*-(3,5-di-*tert*-butyl-2-methoxy-methoxyphenyl)-*L*-cysteine [Boc-*L*-*mod*-Cys(MOM)-OH], 47[89, 95]

II-MAG-33

55   57   47
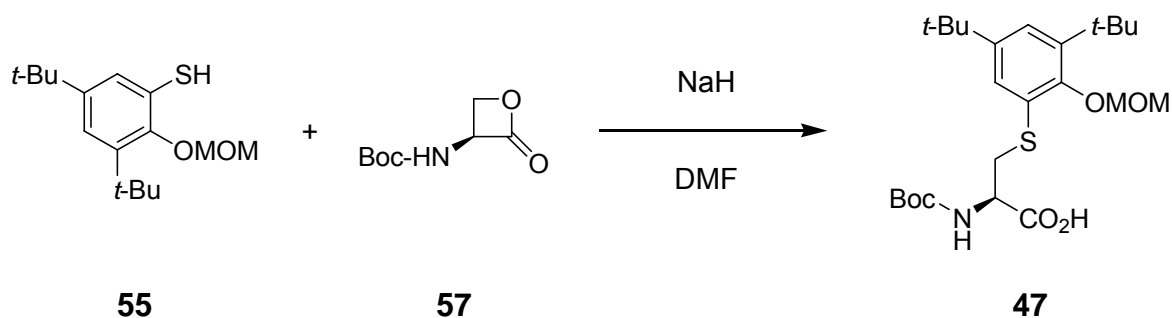
In a dry 500 mL three-necked flask equipped with a condenser, a dropping funnel and under argon, 266 mg (11.1 mmol, 1.05 eq) of sodium hydride were suspended in 50 mL of absolute DMF. To this suspension, a solution of 3.00 g (10.6 mmol, 1.00 eq) of 3,5-di-*tert*-butyl-2-(methoxymethoxy)-benzenethiol **55** in 50 mL of absolute DMF was dropped. The solution was stirred at room temperature under argon for one hour. A solution of 2.10 g (11.1 mmol, 1.05 eq) of *N*-(*tert*-butyloxycarbonyl)-*L*-serine-β-lactone **57** in 125 mL of absolute DMF was added and the reaction mixture was stirred at room temperature under argon for additional 12 hours. The reaction mixture was neutralised by the addition of 100 mL of a 0.05 M hydrochloric solution. The product was extracted with ethyl acetate, washed with water and the organic layer was dried over magnesium sulphate. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (silica gel, *c*-hexane : dichloromethane : methanol = 4 : 4 : 1) to give 4.20 g (9.00 mmol, 85 %) of **47** as a white solid. The product was recrystallised from ethanol.

47

$C_{24}H_{39}NO_6S$ 469.63 g·mol^{-1}

Yield   4.20 g, 9.00 mmol, 85 %

Melting point   61–63 °C (ethanol)

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 2967 [s, ν(aryl–H)], 1718 [s, ν(C=O)], 1662 [s, ν(C=O)], 1540 [m, ν(C=C)], 1516 [s, ν(C=C)], 1404 [m, δ_s(C–H)], 1164 [s, ν(C–O)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.25 (s, 9H, t-Bu), 1.36 (s, 9H, t-Bu), 1.38 (s, 9H, t-Bu), 3.30 (m, 2H, S–CH$_2$), 3.64 (s, 3H, O–CH$_3$), 4.36 (m, 1H, CH), 5.15 (d, ^2J = 4.61, 2H, O–CH$_2$–O), 5.21 (d, ^2J = 4.61, 2H, O–CH$_2$–O), 5.87 (s, 1H, NH), 7.25 (d, $^4J_{Ha,Hb}$ = 2.43, 1H, H$_b$), 7.32 (d, $^4J_{Ha,Hb}$ = 2.43, 1H, H$_a$), 11.3 (s, 1H, CO$_2$H).

^{13}C-NMR   (75 MHz, CDCl$_3$) δ = 27.7 (q, CH$_3$ / t-Bu from Boc), 30.5 (q, CH$_3$ / t-Bu), 30.7 (q, CH$_3$ / t-Bu), 34.1 (s, C$_q$ / t-Bu), 35.0 (s, C$_q$ / t-Bu), 36.3 (t, S–CH$_2$), 53.9 (d, CH), 57.2 (q, O–CH$_3$), 79.7 (s, C$_q$ / O–t-Bu), 99.1 (t, O–CH$_2$–O), 124.0 (d, aryl–C$_6$), 127.8 (s, aryl–C$_1$), 128.8 (d, aryl–C$_4$), 142.5 (s, aryl–C$_3$), 146.2 (s, aryl–C$_5$), 152.9 (s, aryl–C$_2$), 155.8 (s, N–CO), 175.8 (s, COOH).

MS   (negative ESI, MeOH) m/z (%, formula) = 936.89 (10, [dimer]⁻), 468.22 (100, [M − H]⁻), 394.11 (25), 281.25 (10).

TLC R_f = 0.52 (silica gel, *c*-hexane : dichloromethane : methanol = 4 : 4 : 1).

8.3.16 Synthesis of *N*-(benzyloxycarbonyl)-*S*-(3,5-di-*tert*-butyl-2-methoxy-methoxyphenyl)-*L*-cysteine [Cbz-*L*-*mod*-Cys(MOM)-OH], 53[108]

IV-MAG-88

55   **60**   **53**
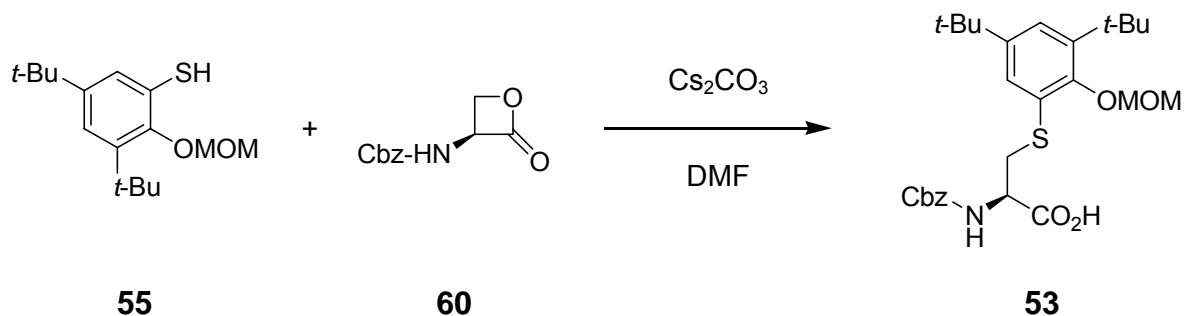
Under argon, a dry 100 mL three-necked flask equipped with a condenser and a dropping funnel was charged with a solution of 652 mg (2.00 mmol, 1.00 eq) of caesium carbonate in 25 mL of absolute DMF. 564 mg (2.00 mmol, 1.00 eq) of 3,5-di-*tert*-butyl-2-(methoxymethoxy)-benzenethiol **55** were added and the solution was stirred at room temperature under argon for one hour. 450 mg (2.00 mmol, 1.00 eq) of *N*-(benzyloxycarbonyl)-*L*-serine-β-lactone **60** were added and the reaction mixture was stirred under argon at room temperature for additional 12 hours. The reaction mixture was neutralised by the addition of 10 mL of a 0.05 M hydrochloric solution. The product was extracted with ethyl acetate, washed with water and the organic layer was dried over magnesium sulphate. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (silica gel, *c*-hexane : dichloromethane : methanol = 4 : 4 : 1) to give 950 mg (1.88 mmol, 94 %) of **53** as a white solid. The product was recrystallised from ethanol.

53
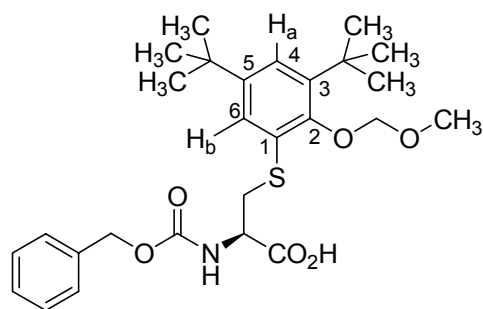
C$_{27}$H$_{37}$NO$_6$S   503.65 g·mol^{-1}

Yield   950 mg, 1.88 mmol, 94 %

Melting point   74 °C (ethanol)

FT-IR   (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 2925 [s, ν(aryl–H)], 1738 [s, ν(C=O)], 1667 [s, ν(C=O)], 1646 [s, ν(C=C)], 1616 [s, ν(C=C)], 1558 [s, ν(C=C)], 1538 [s, ν(C=C)], 1506 [s, ν(C=C)], 1456 [s, ν(C=C)], 1362 [m, δ_s(C–H)], 1162 [s, ν(C–O)], 1082 [s, ν(C–O)], 950 [w, δ_{oop}(aryl–H)], 857 [w, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, CDCl$_3$) δ = 1.25 (s, 9H, t-Bu), 1.37 (s, 9H, t-Bu), 3.11 (dd, ^2J = 13.07, ^3J = 9.01, 1H, S–CH$_2$), 3.44 (dd, ^2J = 13.15, ^3J = 3.50, 1H, S–CH$_2$), 3.53 (s, 3H, O–CH$_3$), 4.06 (td, ^2J = 8.24, ^3J = 3.60, 1H, CH), 5.00 (s, 2H, CH$_2$–Ph), 5.05 (d, ^2J = 4.58, 1H, O–CH$_2$–O), 5.15 (d, ^2J = 4.58, 1H, O–CH$_2$–O), 6.46 (s, 1H, NH), 7.17 (d, $^4J_{Ha,Hb}$ = 2.07, 1H, H$_b$), 7.23 (s, 1H, CH arom. from benzyl), 7.27 (d, $^4J_{Ha,Hb}$ = 2.44, 1H, H$_a$), 7.31 (s, 1H, CH arom. from benzyl), 7.33 (s, 3H, CH arom. from benzyl), 11.2 (s, 1H, CO$_2$H).

¹³C-NMR   (75 MHz, CDCl$_3$) δ = 26.3 (s, C$_q$ / t-Bu), 30.7 (q, CH$_3$ / t-Bu), 31.1
(q, CH$_3$ / t-Bu), 34.3 (s, C$_q$ / t-Bu), 35.0 (t, S–CH$_2$), 54.3 (d, CH),
56.9 (q, O–CH$_3$), 65.2 (t, CH$_2$–Ph), 98.2 (t, O–CH$_2$–O), 121.9 (d,
aryl–C$_6$), 124.8 (d, aryl–C$_4$), 127.6 (d, ortho–PhCH$_2$), 127.7 (d,
para–PhCH$_2$), 128.2 (d, meta–PhCH$_2$), 129.5 (s, aryl–C$_1$), 137.0 (s,
aryl–C$_3$), 141.9 (s, aryl–PhCH$_2$), 145.8 (s, aryl–C$_5$), 151.1 (s, aryl–
C$_2$), 155.7 (s, N–CO), 173.1 (s, COOH).

Specific $[\alpha]_{589}$ = –36.0° (CHCl$_3$, c = 1.00)
rotation $[\alpha]_{546}$ = –43.1° (CHCl$_3$, c = 1.00)
$[\alpha]_{405}$ = –92.0° (CHCl$_3$, c = 1.00)
$[\alpha]_{365}$ = –123.6° (CHCl$_3$, c = 1.00)
$[\alpha]_{334}$ = –163.3° (CHCl$_3$, c = 1.00)

MS   (negative ESI, MeOH) m/z (%, formula) = 1004.69 (50, [dimer]⁻),
722.90 (20), 502.12 (100, [M – H]⁻), 458.11 (35), 394.17 (10).

TLC   R$_f$ = 0.38 (c-hexane : dichloromethane : methanol = 4 : 4 : 1).

8.3.17   Attempted deprotection of the Boc protecting group of Boc-*L-mod*-Cys(MOM)-OH, 47

II-MAG-49

47   76
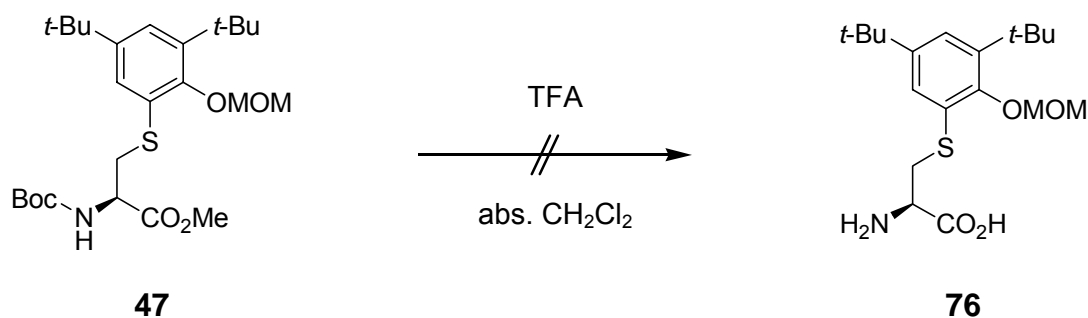
A 5 mL *Schlenk* tube was charged with 50 mg (106 μmol, 1.00 eq) of the *N*-Boc protected Boc-*L*-*mod*-Cys(MOM)-OH **47**, 55.1 μl (742 μmol, 7.00 eq) of TFA and 1 mL of dry dichloromethane. The reaction mixture was stirred at room temperature for 20 minutes. 1 mL of a 0.10 M aqueous sodium hydroxide solution was added to the reaction mixture. The product was extracted with dichloromethane, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. 31.0 mg (95.2 μmol, 90 %) of product **42** were isolated as a white solid.
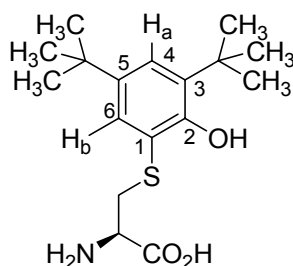
42

C$_{27}$H$_{37}$NO$_6$S   325.47 g·mol^{-1}

Yield   31.0 mg, 95.2 μmol, 90 %

^{1}H-NMR   (300 MHz, d$_6$-DMSO) δ = 1.24 (s, 9H, *t*-Bu), 1.35 (s, 9H, *t*-Bu), 3.18–3.21 (m, 2H, S–CH$_2$), 3.86–3.90 (m, 1H, CH), 7.21 (d, $^4J_{Ha,Hb}$ = 2.35, 1H, aryl–H), 7.38 (d, $^4J_{Ha,Hb}$ = 2.35, 1H, aryl–H).

8.3.18 Attempted deprotection of the Cbz protecting group of Cbz-*L-mod*-Cys(MOM)-OH, 53

IV-MAG-89

53   H₂, Pd/C   MeOH   **76**

Under argon, a 5 mL two-necked flask was charged with 50 mg (99.3 µmol, 1.00 eq) of the *N*-Cbz protected Cbz-*L-mod*-Cys(MOM)-OH **53**, 10 mg (3.38 µmol, 34.0 meq) of palladium catalyst (10 % Pd on charcoal) and 1 mL of dry methanol. The reaction flask was evacuated and filled with dihydrogen (40 bar). The reaction mixture was stirred at room temperature for 3 days. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. 48.0 mg (95.3 µmol, 96 %) of the starting material **53** were re-isolated.

53

C₂₇H₃₇NO₆S   503.65 g·mol⁻¹

Yield   48.0 mg, 95.3 µmol, 96 %

¹H-NMR   (300 MHz, CDCl$_3$) δ = 1.25 (s, 9H, t-Bu), 1.37 (s, 9H, t-Bu), 3.11 (dd, ^2J = 13.07, ^3J = 9.01, 1H, S–CH$_2$), 3.44 (dd, ^2J = 13.15, ^3J = 3.50, 1H, S–CH$_2$), 3.53 (s, 3H, O–CH$_3$), 4.06 (td, ^2J = 8.24, ^3J = 3.60, 1H, CH), 5.00 (s, 2H, CH$_2$–Ph), 5.05 (d, ^2J = 4.58, 1H, O–CH$_2$–O), 5.15 (d, ^2J = 4.58, 1H, O–CH$_2$–O), 6.46 (s, 1H, NH), 7.17 (d, $^4J_{Ha,Hb}$ = 2.07, 1H, H$_b$), 7.23 (s, 1H, CH arom. from benzyl), 7.27 (d, $^4J_{Ha,Hb}$ = 2.44, 1H, H$_a$), 7.31 (s, 1H, CH arom. from benzyl), 7.33 (s, 3H, CH arom. from benzyl), 11.2 (s, 1H, CO$_2$H).

TLC   R$_f$ = 0.38 (c-hexane : dichloromethane : methanol = 4 : 4 : 1).

8.4 Synthesis of two *mod*-Cys-containing decapeptides on amino TentaGel resin

8.4.1 Synthesis of the two decapeptides 78 and 79

II-MAG-45

amino TentaGel: ⬤—[PEG]—NH$_2$ ≡ ⬤—NH$_2$

77

$$\begin{array}{l} H_2N-\mathbf{X_4}-Gly-\mathbf{X_3}-Gly \\ \qquad\qquad\qquad\qquad\qquad \textit{D}\text{-Pro} \\ \text{⬤}-NH-CO-Phe-Gly-\mathbf{X_1}-\mathbf{X_2}-Gly \end{array}$$

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr(*t*-Bu)	X_2 = His(Trt)
X_3 = His(Trt)	X_3 = His(Trt)
X_4 = His(Trt)	X_4 = Tyr(*t*-Bu)
78	**79**
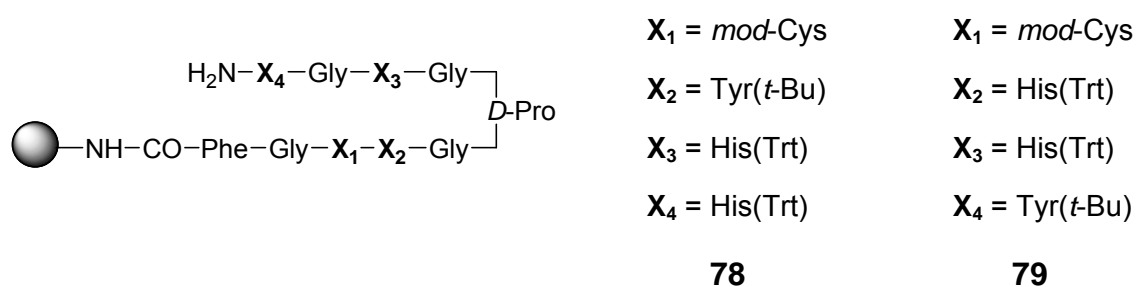
1) swell in DMF

2) 2.50 eq Fmoc-AA-OH, 2.50 eq PyBOP, 3 % NMM in DMF

3) test for free amino functions

4) 20 % piperidine in DMF or 10 % TFA in CH$_2$Cl$_2$

⬤—NH$_2$ ⟶ **78** or **79**

77
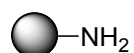
5) repetition of the steps 2) to 4) with the *N*-protected amino acids Fmoc-*L*-Phe-OH, Fmoc-Gly-OH, Boc-*L*-*mod*-Cys(MOM)-OH **47**, Fmoc-*L*-His(Trt)-OH, Fmoc-*L*-Tyr(*t*-Bu)-OH or Fmoc-*D*-Pro-OH according to the given decapeptide sequence

A 100 mL *Merrifield* flask was charged with 200 mg (theoretical loading = 0.25 mmol/g) of amino TentaGel resin **77** and 20 mL of absolute DMF. The resin was allowed to swell by gentle shaking at room temperature for 1 hour. The solvent was filtered and the peptide was synthesised by repetition of the following steps:

1) Coupling: Fmoc-protected amino acids and Boc-protected amino acids were used for the synthesis. The coupling steps followed the same scheme for all amino acids, i.e. coupling in 20 mL of a 3 % NMM solution in DMF with 2.50 eq of the *N*-protected amino acid (Fmoc-*L*-Phe-OH, Fmoc-Gly-OH, Boc-*L*-*mod*-Cys(MOM)-OH **47**, Fmoc-*L*-His(Trt)-OH, Fmoc-*L*-Tyr(*t*-Bu)-OH or Fmoc-*D*-Pro-OH) and 65.0 mg (125 μmol, 2.50 eq) of the coupling agent PyBOP.

2) Test for free amino functions: An NF31 test was performed after each step in order to check the completion of the reaction. In case of a positive test, the coupling step was repeated. In case of a negative test the protecting group was cleaved.

3) Cleavage of the protecting groups: The Fmoc protecting groups were cleaved with 20 mL of a 20 % solution of piperidine in DMF. The Boc protecting groups were cleaved with 20 mL of a 10 % solution of trifluoroacetic acid in dichloromethane. In case of Fmoc-protected amino acids, the coupling step could be quantified with the help of the calibration curve (**Figure 8-1**). In case of Boc-protected amino acids, the coupling step could not be quantified, so a NF31 test was performed after the cleavage in order to check that the deprotection was total. Before execution of the NF31 test, the resin was washed 3 times with a solution of DMF containing 3 % of NMM in order to neutralise the excess in trifluoroacetic acid.

Table 8-3 summarises the amount of amino acids used, as well as the yields for each coupling step.

Table 8-3: Quantity of amino acid used and yields for the coupling steps in the synthesis of peptide 78 and peptide 79.

peptide **78**			peptide **79**		
amino acid[a)]	loading	yield	amino acid[a)]	loading	yield[c)]
[mg]	[mmol/g]	[%]		[mg]	[mmol/g]	[%]
Phe	48.0	0.18	100	Phe	48.0	0.18	100
Gly	37.0	0.17	94	Gly	37.0	0.17	94
mod-Cys	59.0	n.d.	n.d.	*mod*-Cys	59.0	n.d.	n.d.
Tyr[b)]	57.0	0.11	61	His[b)]	77.0	0.18	100
Gly	37.0	0.14	78	Gly	37.0	0.16	89
D-Pro	42.0	0.16	89	*D*-Pro	42.0	0.17	94
Gly[b)]	37.0	0.12	94	Gly[b)]	37.0	0.20	111
His	77.0	0.12	94	His	77.0	0.17	94
Gly	37.0	0.09	50	Gly	37.0	0.12	67
His	77.0	0.08	44	Tyr	57.0	0.11	61

[a)] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

[b)] The coupling step was repeated.

[c)] The values are relative to the first value which was fixed to 100 %.

8.4.2   Acetylation of the *N*-terminus of the decapeptides

II-MAG-53

78

$H_2N-\textbf{X}_4-Gly-\textbf{X}_3-Gly\rceil$

⬤—NH−CO−Phe−Gly−$\textbf{X}_1$−$\textbf{X}_2$−Gly⌐ *D*-Pro

79

$\textbf{X}_1$ = *mod*-Cys

$\textbf{X}_2$ = Tyr(*t*-Bu)

$\textbf{X}_3$ = His(Trt)

$\textbf{X}_4$ = His(Trt)

1) Ac₂O, 3 % NMM in DMF

2) test for free amino functions

$\textbf{X}_1$ = *mod*-Cys

$\textbf{X}_2$ = His(Trt)

$\textbf{X}_3$ = His(Trt)

$\textbf{X}_4$ = Tyr(*t*-Bu)

80

$H_3C-C(=O)-\underset{H}{N}-\textbf{X}_4-Gly-\textbf{X}_3-Gly\rceil$

⬤—NH−CO−Phe−Gly−$\textbf{X}_1$−$\textbf{X}_2$−Gly⌐ *D*-Pro

81
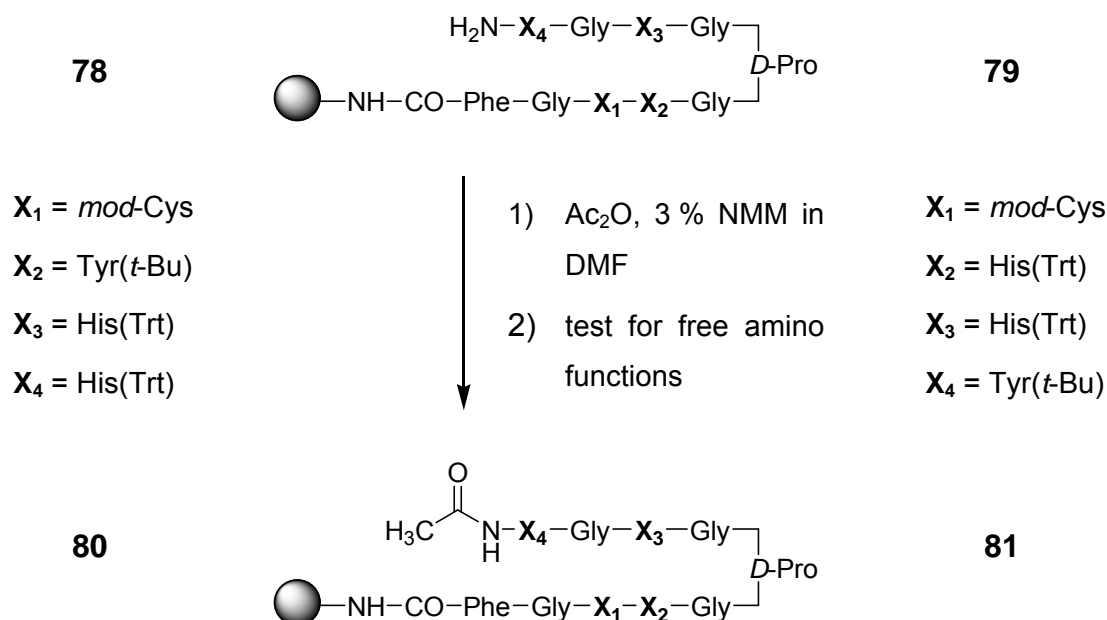
20 mL of a 3 % NMM solution in absolute DMF were added to the resin (200 mg, 50.0 μmol, 1.00 eq). 12.0 μL (125 μmol, 2.50 eq) of acetic anhydride were added to the suspension and the reaction mixture was shaken at room temperature under argon for 12 hours. The reaction mixture was filtered and the resin washed 3 times with DMF. A NF31 test was carried out on a few beads of **80** and **81** in order to check the completion of the reaction. The colour test was negative, indicating that the reaction had been successful. By comparison, the test performed on the starting material **78** and **79** was positive.

8.4.3 Cleavage of the side chain protecting groups

II-MAG-54
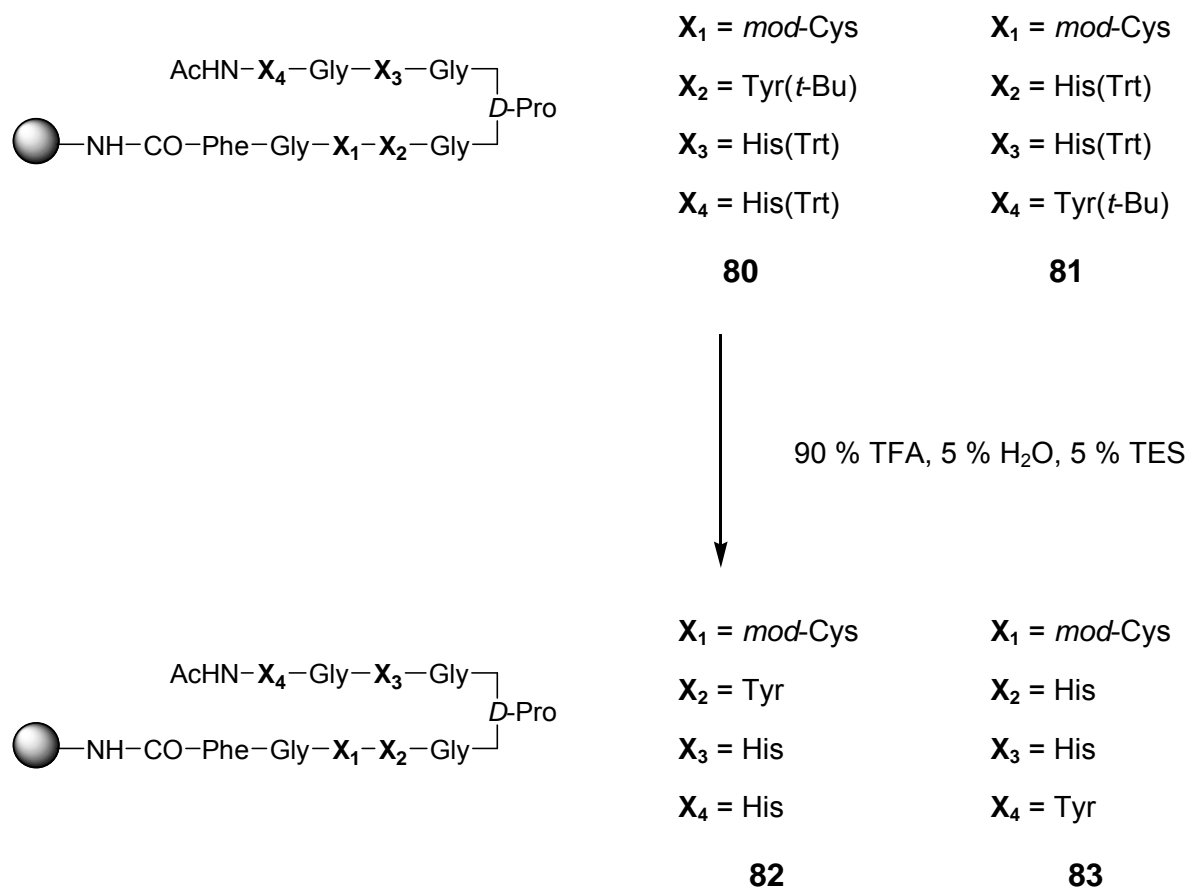
AcHN−X_4−Gly−X_3−Gly⌐
  ⌐D-Pro
●—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr(*t*-Bu)	X_2 = His(Trt)
X_3 = His(Trt)	X_3 = His(Trt)
X_4 = His(Trt)	X_4 = Tyr(*t*-Bu)
80	**81**

90 % TFA, 5 % H_2O, 5 % TES

AcHN−X_4−Gly−X_3−Gly⌐
  ⌐D-Pro
●—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_1 = *mod*-Cys	X_1 = *mod*-Cys
X_2 = Tyr	X_2 = His
X_3 = His	X_3 = His
X_4 = His	X_4 = Tyr
82	**83**

The resin was washed 3 times with dichloromethane, then 3 times with methanol in order to remove the DMF. The peptides were shaken in 20 mL of a mixture of 90 % TFA, 5 % water and 5 % triethylsilane at room temperature for 5 hours. The procedure was repeated to ensure total deprotection of the side chains. The yellow solution was filtered and the resin was washed successively with dichloromethane, methanol and water.

8.4.4 Formation of the peptide–copper(II) complexes

II-MAG-55
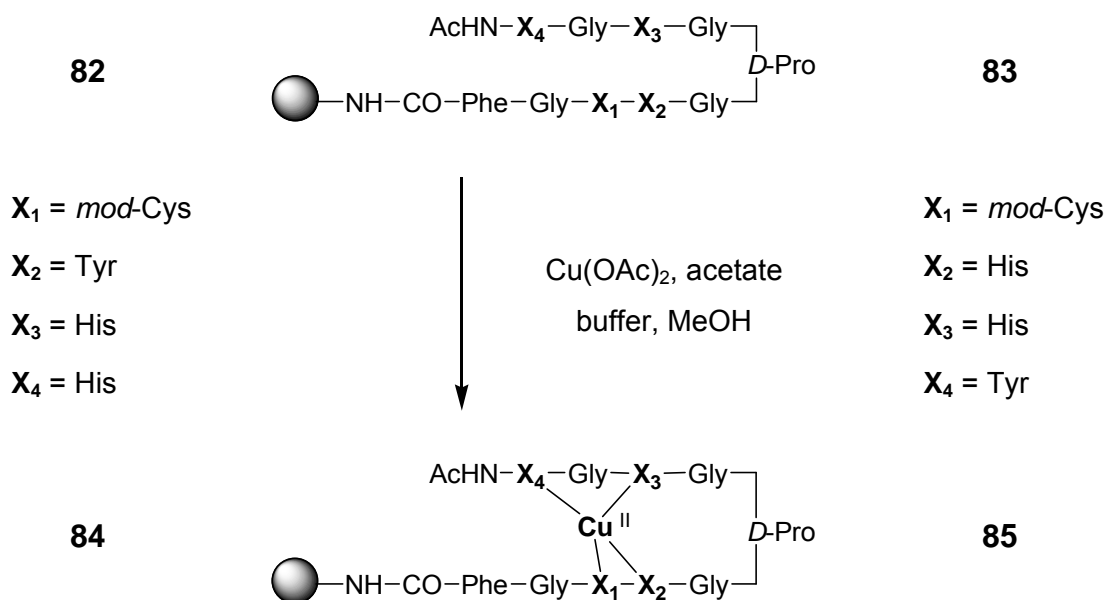
AcHN–**X$_4$**–Gly–**X$_3$**–Gly⌐
82   ⬤—NH–CO–Phe–Gly–**X$_1$**–**X$_2$**–Gly—   *D*-Pro   **83**

X$_1$ = *mod*-Cys   **X$_1$** = *mod*-Cys

X$_2$ = Tyr   Cu(OAc)$_2$, acetate   **X$_2$** = His

X$_3$ = His   buffer, MeOH   **X$_3$** = His

X$_4$ = His   **X$_4$** = Tyr

AcHN–**X$_4$**–Gly–**X$_3$**–Gly⌐
84   ⬤—NH–CO–Phe–Gly–**X$_1$**–**X$_2$**–Gly—   **Cu**^{II} *D*-Pro   **85**

200 mg (50.0 μmol, 1.00 eq) of the solid-supported peptide-ligands were washed twice with methanol. Then 20 mL of methanol, 9.10 mg (50.0 μmol, 1.00 eq) of copper(II) acetate, 115 μL of acetic acid and 164 mg of sodium acetate (100 mM acetate buffer) were added to the resin. The reaction mixture was shaken at room temperature under argon for 5 hours and the resin was washed 3 times with methanol. The colour of the beads before and after complexation was compared. While the peptide–ligands had a pale yellow colour, the complexes had a green–blue colour.

8.5 Oxidation of 3-methoxybenzyl alcohol 39 catalysed by the *mod*-Cys containing peptide–copper(II) complexes 84 and 85

8.5.1 Catalysis with solvent

II-MAG-58

39 → **40**

peptide–copper(II) complex **84** or **85**, KOH, O$_2$

MeCN / H$_2$O (1:1), r.t.
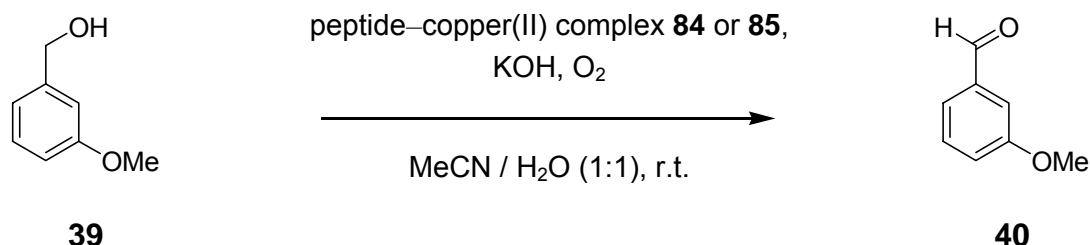
A 10 mL round-bottomed flask was charged with 4.50 μL (36.0 μmol, 7.00 mM, 1.00 eq) of 3-methoxybenzyl alcohol **39**, 1.00 mg (0.18 μmol, 0.50 mol%) of peptide–copper(II) complex **84** or **85**, 0.10 mg (1.80 μmol, 5.00 mol%) of potassium hydroxide and 5 mL of a mixture of acetonitrile and water (1:1). Under an atmosphere of dioxygen, the reaction mixture was shaken at room temperature for 24 hours. The reaction was followed by measuring the UV absorbance at $\lambda = 310$ nm of 250 μL samples. The results are summarised in **Table 8-4**.

Table 8-4: 3-methoxybenzaldehyde 40 formed after 24 hours by catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39 with peptide–copper(II) complexes 84 and 85.

peptide–copper(II) complex	KOH	3-methoxybenzaldehyde	yield	TON
	[mol%]	[mol%]	[μmol]	[%]	
-	-	-	0.14	0.39	<1
-	-	5.00	0.36	1.01	2.0
84	0.50	-	0.15	0.41	<1
85	0.50	-	0.13	0.36	<1
84	0.50	5.00	0.30	0.83	1.7
85	0.50	5.00	0.38	1.06	2.1

8.5.2 Catalysis without solvent

II-MAG-68
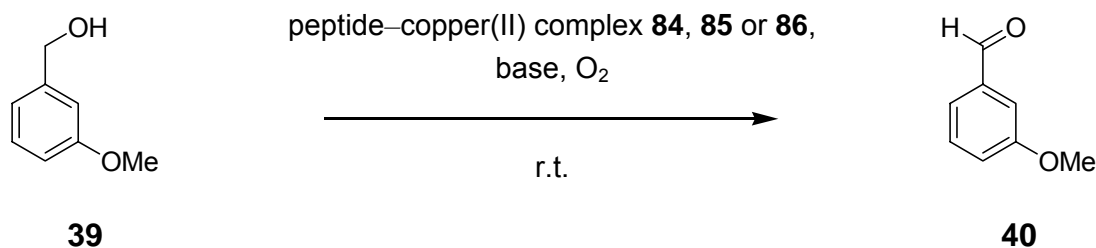
39 → **40**

20.0 μL (161 μmol, 1.00 eq) of 3-methoxybenzyl alcohol **39** were mixed with 1.00 mg (0.18 μmol, 0.10 mol%) of peptide–copper(II) complex **84** or **85**, as well as 2.00 mg (35.7 μmol, 20.0 mol%) of potassium hydroxide or 5.00 μL (35.6 μmol, 20.0 mol%) of triethylamine. Under an atmosphere of dioxygen, the reaction mixture was shaken at room temperature for 24 hours. To check the progress of the reaction, aliquots were taken with a pipette, filtered and diluted in dichloromethane for GC analysis (GC program 1).

For comparison, 20.0 μL (161 μmol, 1.00 eq) of 3-methoxybenzyl alcohol **39** were mixed with 1.00 mg (0.18 μmol, 0.10 mol%) of acetylated amino TentaGel **86**, 33 μg (0.18 μmol, 0.10 mol%) of copper(II) acetate and 2.00 mg (35.7 μmol, 20.0 mol%) of potassium hydroxide. The reaction was performed and followed in the same conditions as above.

acetylated amino TentaGel:

86
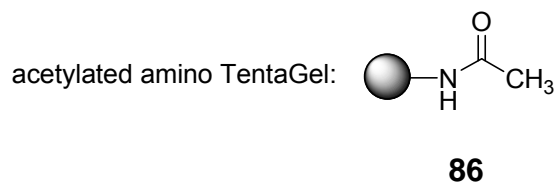
Table 8-5: Influence of the base on the catalytic aerobic oxidation of neat 3-methoxybenzyl alcohol 39 with peptide–copper(II) complexes 84 and 85 after 24 hours.

catalyst		base		3-methoxybenzaldehyde[a)]	yield	TON
	[mol%]		[mol%]	[μmol]	[%]	
84	0.10	-	-	-	-	-
85	0.10	-	-	-	-	-
84	0.10	NEt$_3$	20.0	-	-	-
85	0.10	NEt$_3$	20.0	-	-	-
-	-	KOH	20.0	0.10	0.06	<1
86	0.10	KOH	20.0	1.93	1	10
84	0.10	KOH	20.0	6.28	4	40
85	0.10	KOH	20.0	4.51	3	30

[a)] 3-Methoxybenzoic acid was not formed.

8.6 Synthesis of *N*-(9-fluorenylmethoxycarbonyl)-2,2,6,6-tetramethyl-piperidine-1-oxyl-4-amino-4-carboxylic acid (Fmoc-TOAC-OH), 43

8.6.1 Synthesis of 2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl, 88[93]

III-MAG-32

87 → **88**
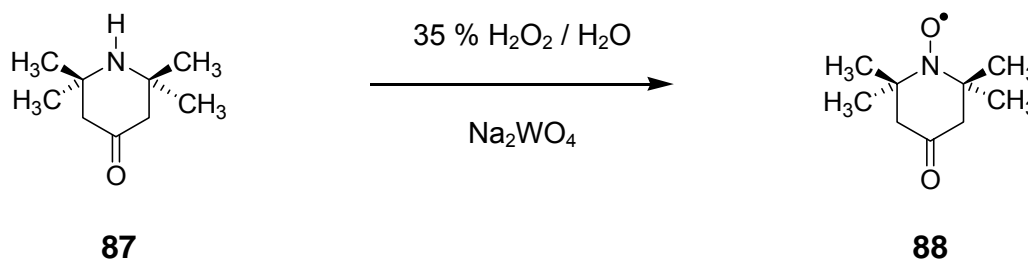
In a 500 mL round-bottomed flask, a solution of 50.0 g (322 mmol, 1.00 eq) of 2,2,6,6-tetramethylpiperidin-4-one **87** and 5.50 g (16.7 mmol, 0.05 eq) of sodium tungstate dihydrate in 300 mL of water was cooled down to 5 °C. 80 mL of a 35 %

aqueous solution of hydrogen peroxide were added with stirring and the suspension was stirred vigorously at room temperature for 16 hours. The yellow solution turned orange. The solution was saturated with potassium carbonate and the product was extracted with diethylether. The organic layer was dried over magnesium sulphate and the solvent was evaporated under reduced pressure to give an orange solid that was recrystallised from *n*-hexane. 50.8 g (297 mmol, 93 %) of the desired product **88** were obtained as orange needles.
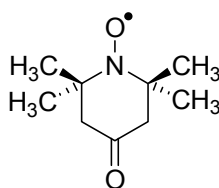
88

$C_9H_{16}NO_2\cdot$	170.23 g·mol^{-1}
Yield	50.8 g, 298 mmol, 93 % (Lit.: 88 %)[93]
Melting point	46 °C (Lit.: 36–37 °C)
FT-IR	(CsI disc) $\tilde{v}$ [cm^{-1}] = 2982 [m, v(alkyl–C–H)], 1720 [s, v(C=O)], 1468 [m, δ_{as}(C–H)], 1410 [m, δ_s(C–H)], 1365 [m, δ_{as}(C–H from CH$_3$)], 1325 [m, δ_s(C–H from CH$_3$)], 1239 [s].
MS	(CH$_2$Cl$_2$) m/z (formula) = 170 ([M$^+$]), 114 ([M − 4×CH$_2$]$^+$).
TLC	R$_f$ = 0.28 (silica gel, dichloromethane).

X-ray	temperature	100(2) K
	wavelength	0.71073 Å
	crystal system, space group	monoclinic, $C\,2/c$
	unit cell dimensions	a = 35.3622(10) Å, α = 90°
		b = 5.95880(10) Å, β = 99.4690(10)°
		c = 27.9559(10) Å, γ = 90°
	volume	5810.5(3) Å^3
	Z	24
	calculated density	1.168 g/cm^3
	absorption coefficient	0.082 mm^{-1}
	F(000)	2232
	crystal size	0.05 x 0.2 x 0.3 mm
	Θ range	1.48° until 27.00°
	limiting indices	$-$43 < h < 44
		$-$6 < k < 7
		$-$35 < l < 35
	reflections collected / unique	11363 / 6308 [R(int) = 0.0646]
	reflection observed [I>2σ(I)]	3780
	refinement method	full-matrix least squares on F^2
	data / restraints / parameters	6003 / 6 / 451
	goodness-of-fit on F^2	0.983
	R indices [I>2σ(I)]	R1 = 0.0526, wR2 = 0.1286
	R indices (all data)	R1 = 0.0982, wR2 = 0.1497
	largest diff. peak and hole	0.324 and $-$0.446 eÅ^{-3}

8.6.2 Synthesis of 2,2,6,6-tetramethyl-2',5'-dioxopiperidine-4-spiro-4-imidazo lidine-1-oxyl, 89[93, 124]

III-MAG-32

88 → **89**

(NH₄)₂CO₃, NaCN — EtOH : H₂O (3:2), 60 °C
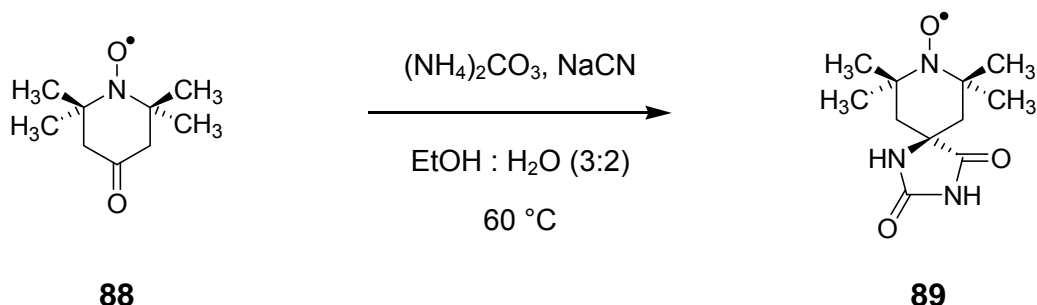
In a 250 mL three-necked flask equipped with a reflux condenser, 44.0 g (258 mmol, 1.00 eq) of 2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl **88** and 98.9 g (1.03 mol, 4.00 eq) of ammonium carbonate were dissolved in 150 mL of a 3:2 mixture of ethanol and water. A solution of 25.3 g (516 mmol, 2.00 eq) of sodium cyanide in 60 mL of water was added with stirring. The reaction mixture was stirred and heated to 60°C for 20 hours. The colour of the solution turned from red to orange. The product was extracted with ethyl acetate, dried over magnesium sulphate and the solvent was evaporated under reduced pressure to give 51.4 g (214 mmol, 83 %) of **89** as an orange solid.

89
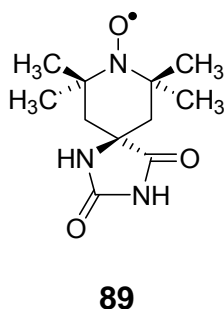
$C_{11}H_{18}N_3O_3\cdot$ 240.28 g·mol^{-1}

Yield   51.4 g, 214 mmol, 83 % (Lit.: 95 %)[93]

Melting point   255 °C (dec.), (Lit.: 272 °C)[124]

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3169 [br, ν(N–H)], 1775 [m, ν(C=O)], 1722 [s, ν(C=O)], 1414 [m, δ(C–H)], 1366 [m, δ(C–H from CH$_3$)], 1243 [m], 1037 [m], 768 [w], 670 [w], 455[w].

MS   (CH$_2$Cl$_2$) m/z (formula) = 240 ([M$^+$]), 226 ([C$_{10}$H$_{16}$N$_3$O$_3$$^+$]), 210 ([C$_9H_{12}N_3O_3$$^{2+}$]), 154, 139.

TLC R_f = 0.43 (silica gel, dichloromethane : methanol = 19 : 1).

8.6.3   Synthesis of 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC), 41[93, 124]

III-MAG-32

89 → **41**
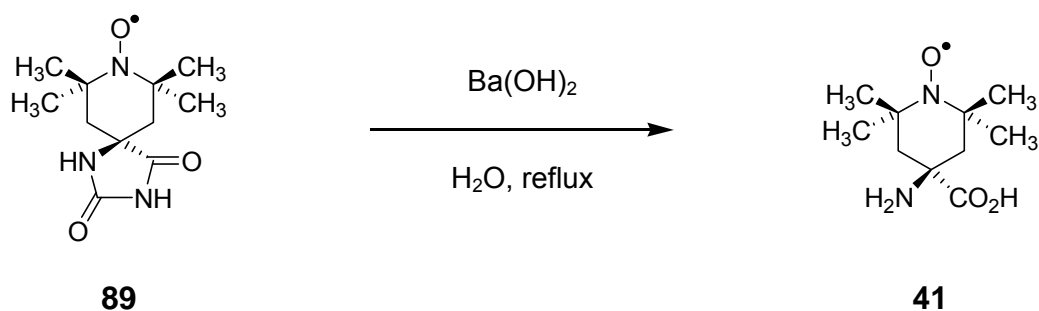
In a 2 L round-bottomed flask equipped with a reflux condenser, 40.0 g (166 mmol, 1.00 eq) of 2,2,6,6-tetramethyl-2',5'-dioxopiperidine-4-spiro-4-imidazolidine-1-oxyl **89** and 210 g (666 mmol, 4.00 eq) of barium hydroxide octahydrate were dissolved in 800 mL of water. The reaction mixture was heated to reflux for 48 hours. It was then saturated with dry ice and filtered. The filtrate was acidified to pH 6–7 with hydrochloric acid and the solvent was evaporated under reduced pressure to give the

crude product which was recrystallised from a 80 % aqueous ethanol solution. 31.5 g (146 mmol, 88 %) of the product **41** were obtained as a yellow solid.
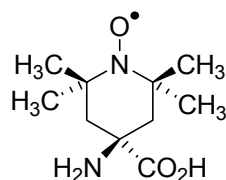
41

$C_{10}H_{19}N_2O_3$·   215.27 g·mol^{-1}

Yield   31.5 g, 146 mmol, 88 % (Lit.: 71 %)[93]

Melting point   225–230 °C (dec.) (Lit.: 228–230 °C)[124]

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3151 [m, v(N–H)], 3046 [m, v(O–H)], 1620 [s, v(C=O)], 1527 [s], 1463 [m], 1403 [s, δ(C–H from CH$_3$)], 1354 [m, δ(C–H from CH$_3$)], 1321 [s, δ(C–H)], 1225 [m, δ(C–H)], 1175 [m], 1065 [m], 910 [w], 822 [m], 788 [w], 555 [w], 416 [m].

8.6.4   Synthesis of *N*-(9-fluorenylmethoxycarbonyl)-2,2,6,6-tetramethyl-piperidine-1-oxyl-4-amino-4-carboxylic acid (Fmoc-TOAC-OH), 43[116]

IV-MAG-82

Fmoc-OSu, NaHCO$_3$

1,4-dioxane, H$_2$O
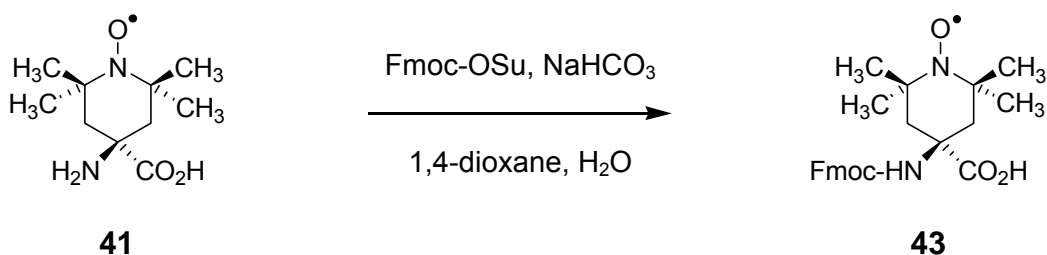
41   **43**

In a 250 mL round-bottomed flask, 5.50 g (25.5 mmol, 1.00 eq) of TOAC **41** and 2.10 g (25.5 mmol, 1.00 eq) of sodium bicarbonate were suspended in 80 mL of water. A solution of 12.9 g (38.3 mmol, 1.50 eq) of *N*-(9-fluorenylmethoxy-carbonyloxy)-succinimide (Fmoc-OSu) in 50 mL of 1,4-dioxane was added. The reaction mixture was stirred at room temperature in the darkness for 3 days. The pH was brought to 8 by the addition of sodium bicarbonate. The 1,4-dioxane was evaporated under reduced pressure and the unreacted Fmoc-OSu was extracted from the remaining aqueous phase with diethylether. The pH was adjusted to 3 with potassium bisulphate and the product was extracted with ethyl acetate. The organic layer was washed with a 10 % potassium bisulphate solution, then with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The product **43** was formed together with the hydroxylamine **90**. They could be separated by chromatography (silica gel, dichloromethane : methanol = 19 : 1) to yield 6.20 g (14.2 mmol, 56 %) of the pure product **43** as a yellow solid which could be crystallised from a mixture of ethyl acetate and *n*-hexane (1:1) to give small yellow needles.
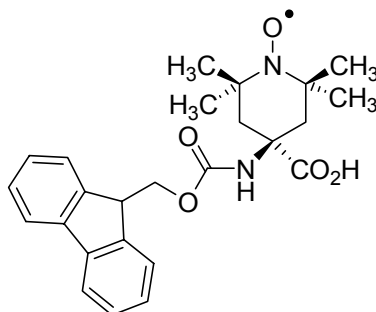
43

C$_{25}$H$_{29}$N$_2$O$_5$·	437.51 g·mol^{-1}
Yield	6.20 g, 14.2 mmol, 56 % (Lit.: 62 %)[116]
Melting point	165 °C (Lit.: 161–163 °C)[116]

FT-IR (CsI disc) $\tilde{\nu}$ [cm^{-1}] = 3342 [s, ν(N–H)], 2986 [m, ν(alkyl–C–H)], 1724 [s, ν(C=O)], 1722 [s, ν(C=O)], 1529 [s], 1474 [w, δ(C–H)], 1451 [m, δ(C–H)], 1374 [w, δ(C–H)], 1294 [m], 1244 [s, ν(C–O)], 1242 [s, ν(C–O)], 1172 [s, ν(C–O)], 1108 [s, ν(C–O)], 1032 [w], 741 [s, δ_{oop}(aryl–H)], 692 [m, δ_{oop}(aryl–H)].

MS (positive ESI, DMSO / MeOH) m/z (formula) = 439 ([M + 2 × H]$^+$).

HR-MS (positive ESI)

	measured	calculated [M + 2 × H]$^+$	$\vert\Delta mu\vert$
	439.223	439.2233	0.005

TLC R_f = 0.62 (silica gel, dichloromethane : methanol = 19 : 1).

X-ray A X-ray structure was obtained (**Figure 5-11**). However, the quality of the crystals did not allow the computation of the data.
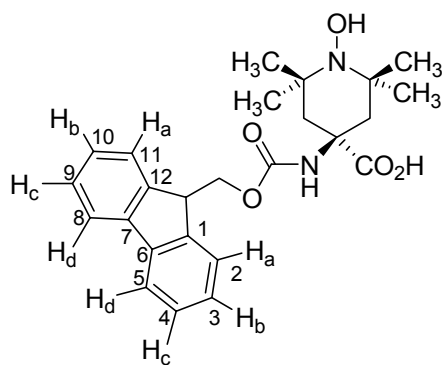
90

C$_{25}$H$_{30}$N$_2$O$_5$ 438.52 g·mol^{-1}

Yield   3.10 g, 7.10 mmol, 28 %

FT-IR   (CsI disc) $\tilde{v}$ [cm^{-1}] = 3412 [br, ν(O–H)], 3069 [m, ν(aryl–H)], 2993 [m, ν(alkyl–C–H)], 1727 [s, ν(C=O)], 1606 [s, ν(C=O)], 1501 [s, ν(C=C)], 1451 [s, δ_{as}(C–H)], 1387 [s, δ_{s}(C–H)], 1251 [s, ν(C–O)], 1183 [m, ν(C–O)], 1118 [m, ν(C–O)], 1051 [m, ν(C–O)], 744 [m, δ_{oop}(aryl–H)], 742 [s, δ_{oop}(aryl–H)], 741 [s, δ_{oop}(aryl–H)], 656 [w, δ_{oop}(aryl–H)], 622 [m, δ_{oop}(aryl–H)], 589 [m, δ_{oop}(aryl–H)], 544 [w, δ_{oop}(aryl–H)].

^{1}H-NMR   (300 MHz, DMSO) δ = 1.05 (s, 6H, CH$_3$), 1.18 (s, 6H, CH$_3$), 1.92 (m, 2H, CH$_2$), 2.36 (m, 2H, CH$_2$), 4.20–4.24 (m, 3H, CH+CH$_2$ from Fmoc), 7.30 (dd, $^3J_{Ha,Hb}$ = 7.40, $^3j_{Hb,Hc}$ = 6.20, 2H, H$_b$), 7.40 (dd, $^3J_{Hc,Hd}$ = 7.42 , $^3J_{Hb,Hc}$ = 6.91, 2H, H$_c$), 7.70 (d, $^3J_{Ha,Hb}$ = 7.46, 2H, H$_a$), 7.87 (d, $^3J_{Hc,Hd}$ = 7.31, 2H, H$_d$).

^{13}C-NMR (75 MHz, DMSO) δ = 25.2 (q, CH$_3$), 41.6 (t, CH$_2$), 45.9 (d, CH–Fmoc), 71.8 (t, CH$_2$–Fmoc), 120.2 (d, aryl–C$_5$ + C$_8$), 124.8 (d, aryl–C$_2$ + C$_{11}$), 127.2 (d, aryl–C$_3$ + C$_{10}$), 127.9 (d, aryl–C$_4$ + C$_9$), 140.7 (s, aryl–C$_6$ + C$_7$), 142.5 (s, aryl–C$_1$ + C$_{12}$), 151.0 (s, N–CO), 169.6 (s, COOH).

TLC R_f = 0.60 (silica gel, dichloromethane : methanol = 19 : 1).

8.7 Synthesis of the ligand library of the general scheme Ac-NH-X-Gly-X-Gly-*D*-Pro-Gly-X-X-Gly-Phe-CO-NH-TentaGel (TOAC library)

8.7.1 Synthesis of the tripeptide H₂N-TOAC-Gly-Phe-CO₂H 92 on *Wang* resin and cleavage from the resin

V-MAG-5

8.7.1.1 Synthesis of the tripeptide **92** on *Wang* resin
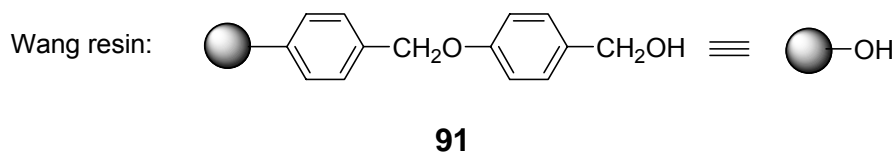
Wang resin:

91

1) swell in DMF
2) 5.00 eq Fmoc-*L*-Phe-OH, 5.00 eq DIC, 5.00 eq HOBt, 0.10 eq DMAP
3) test for free amino functions
4) 20 % piperidine in DMF
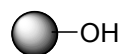
5) 2.50 eq Fmoc-Gly-OH, 2.50 eq PyBOP, 3 % NMM in DMF
6) repetition of the steps 3) and 4), then 5) with Fmoc-TOAC-OH and finally 3) and 4)
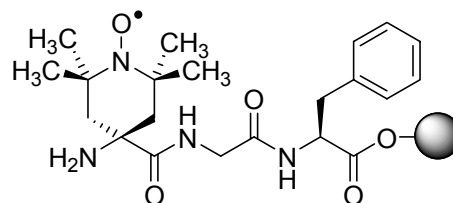
91   **92**

A 100 mL *Merrifield* flask was charged with 100 mg (theoretical loading = 0.97 mmol/g) of *Wang* resin and 10 mL of a mixture of dry DCM and absolute DMF (8:2). The resin was allowed to swell by gentle shaking at room temperature for 1 hour. The *Wang* resin was loaded with Fmoc-*L*-Phe-OH according to the general procedure (**8.2.9**). The loading of the resin was determined to be 1.10 mmol/g. The second and third amino acid (Fmoc-Gly-OH and Fmoc-TOAC-OH) were coupled according to the standard PyBOP / Fmoc protocol (**8.2.4** and **8.2.6**).

NF31 colour tests indicated the completion of each coupling step. The yields for each coupling step determined by UV-quantification of the dibenzofulvene–piperidine adduct are summarised in **Table 8-6**.

Table 8-6: **Coupling yields for the synthesis of the tripeptide 92 on *Wang* resin.**

amino acid	loading [mmol/g]	coupling [%]
Phe [a)	1.10	91
Gly	1.21	100
TOAC	0.75	68

[a) *L*-amino acid

8.7.1.2 Cleavage of the tripeptide **92** from the *Wang* resin

92   **93**
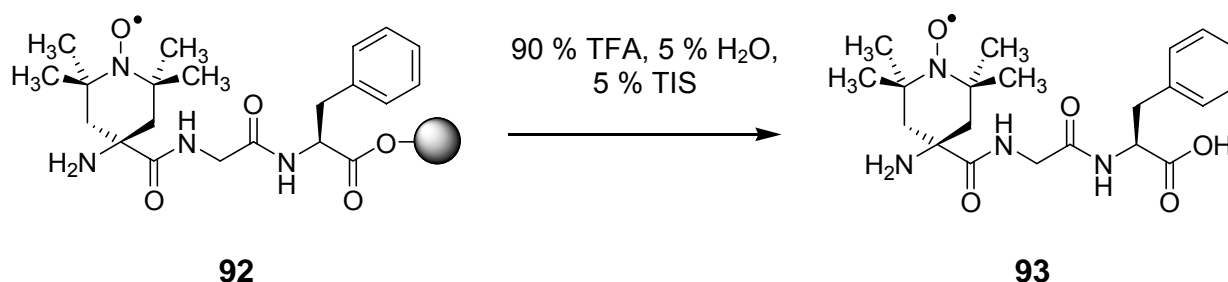
The resin loaded with the tripeptide **92** was washed 3 times with dichloromethane and 3 times with methanol in order to remove the DMF. It was shaken 5 hours in 10 mL of a mixture of 90 % of TFA, 5 % of water and 5 % of triisopropylsilane. The treatment with TFA, water and triisopropylsilane was repeated for another period of 5 hours. The yellow solution was filtered and the filtrate was concentrated under reduced pressure. The product precipitated by addition of diethylether. The product was filtered, dissolved in water and lyophilised to give 38 mg (90.6 μmol, 83 %) of a white powder. The crude product was analysed by LC–MS.

	93	94	95

$C_{21}H_{31}N_4O_5 \cdot$ $C_{21}H_{32}N_4O_5$ $C_{12}H_{22}N_3O_4 \cdot$

419.23 g·mol^{-1} 420.50 g·mol^{-1} 272.32 g·mol^{-1}
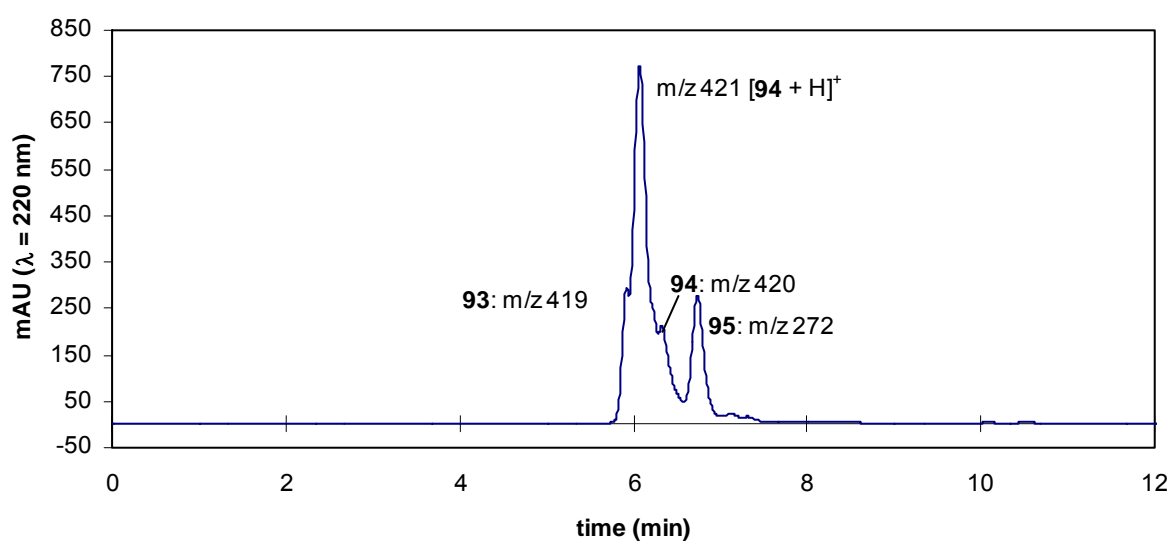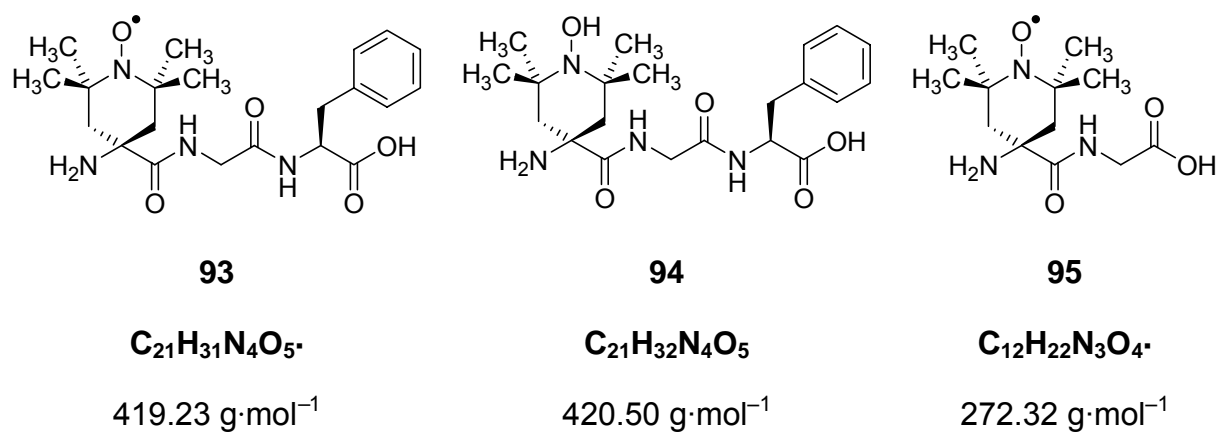
Figure 8-2: Baseline corrected LC–MS diagram of the crude product (column: RP-18, mobile phase: MeCN : H$_2$O : AcOH = 90 : 10 : 0.5).

8.7.2 Combinatorial synthesis of the 81mer library of decapeptides (TOAC library)[40, 41, 125]

III-MAG-76

amino TentaGel:
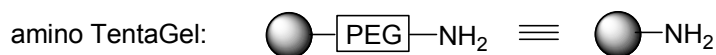
77
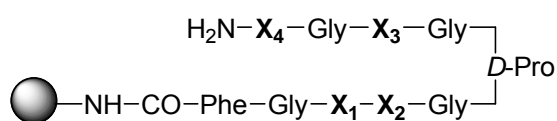
X_n = His(Trt), Tyr(*t*-Bu), TOAC

96

1) swell in DMF

2) 2.50 eq Fmoc-*L*-Phe-OH, 2.50 eq PyBOP, 3 % NMM in DMF

3) test for free amino functions

4) 20 % piperidine in DMF

77
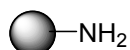
5) repetition of the steps 2) to 4) with the Fmoc-protected amino acids, Fmoc-Gly-OH, Fmoc-*L*-His(Trt)-OH, Fmoc-*L*-Tyr(*t*-Bu)-OH, Fmoc-TOAC-OH **43** or Fmoc-*D*-Pro-OH, following the split-mix methodology

96

81 *IRORI* MikroKans™ were filled each with 30.0 mg (theoretical loading = 0.26 mmol/g) of amino TentaGel **77**. Each MikroKan was equipped with a radiofrequency *IRORI* AccuTag™ which was programmed with a code, each code corresponding to a specific sequence of amino acids. The code of the AccuTag could be read at any time during the synthesis of the peptides. The MicroKans were placed in a flask containing 100 mL of absolute DMF, and the resin was allowed to swell by gentle shaking of the flask at room temperature under argon for 2 hours.

The synthesis of the library was accomplished by repetitions of the 3 following steps: coupling, test for free amino functions, cleavage. The split-mix method was followed, i.e. after each step the AccuTags were read with the *IRORI* AccuTag scanning station and divided in different flasks in order to couple on each peptide the corresponding amino acid.

1) Coupling: A 3 % solution of NMM in absolute DMF, 2.50 eq of PyBOP and 2.50 eq of the corresponding amino acid were added to the resin. The reaction mixture was shaken at room temperature under argon for 12 hours. In case of coupling of the amino acids following TOAC **43**, as well as for the last amino acids of the sequences, the coupling solution was filtered and the coupling was repeated with fresh reagents and shaken for an additional period of 6 hours. The solution was then filtered and the resin washed 3 times with DMF.

2) Test for free amino functions, NF31 test: A few beads were taken out of one of the MikroKans™ and transferred into a vial, 1.00 mL of a 0.01 mM solution of NF31 in acetonitrile was added and the reaction mixture was warmed to 70 °C for 10 minutes. The solution was filtered and the beads were washed 3 times with each of the following solvents: DMF, methanol and dichloromethane. The test was considered negative if the beads were colourless after washing; in this case the synthesis was pursued with stage 3). In the case of a positive test (red beads), the coupling step was repeated.

3) Cleavage of Fmoc protecting groups: The MikroKans™ were shaken at room temperature under argon for 2 hours in a 20 % solution of piperidine in absolute DMF. The treatment with piperidine was repeated 2 more times for one hour. The solution was filtered and the resin was washed 3 times with absolute DMF. The solutions were collected, combined and the UV absorbance at $\lambda = 300$ nm was measured in order to calculate the yield of the coupling.
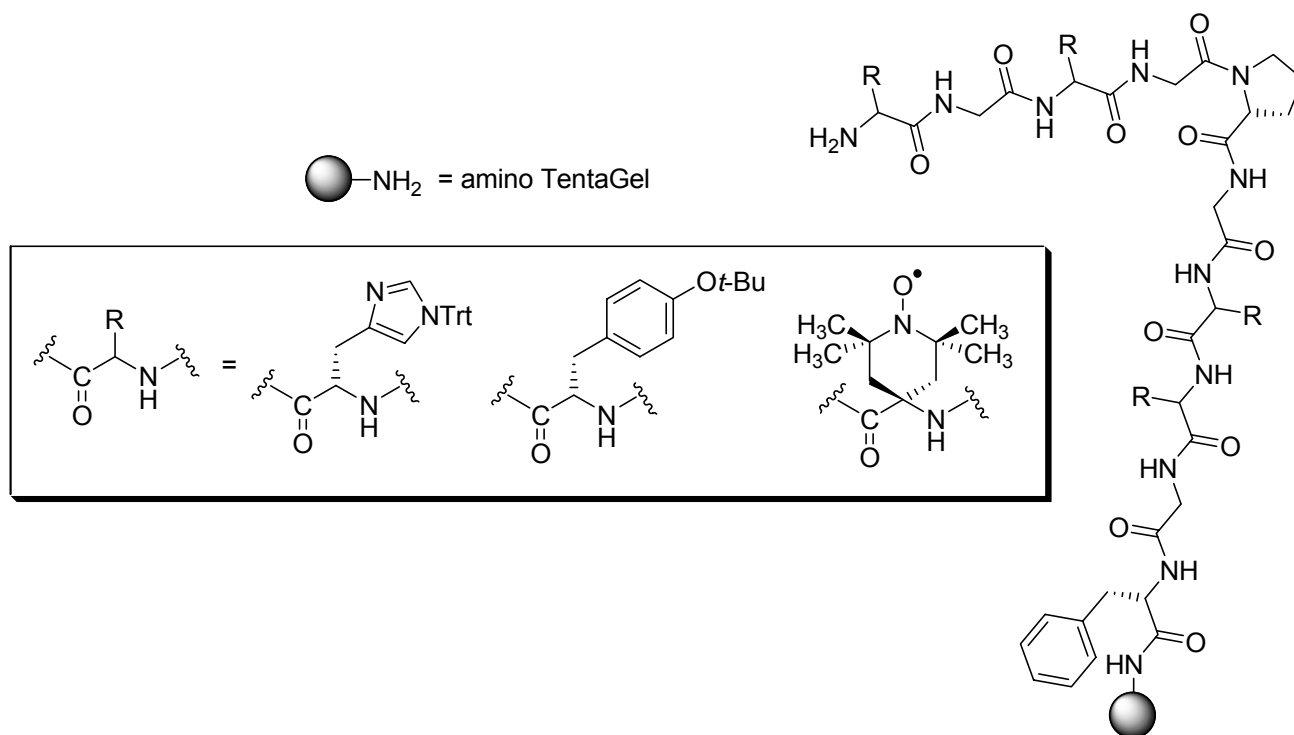
96

The quantities of Fmoc protected amino acids and PyBOP used for each step are summarised in **Table 8-7**, together with the yield of each coupling step (**Figure 8-3**).

Table 8-7: **Synthesis of the 81-member peptide library. Quantities of amino acid and PyBOP used, yield for each coupling step.**

coupling	amino acid[a)]			PyBOP		loading	yield
		[mg]	[mmol]	[mg]	[mmol]	[mmol/g]	[%]
1	Phe	612	1.58	822	1.58	0.218	100
2	Gly	470	1.58	822	1.58	0.212	97
3a	His	326	0.53	274	0.53	0.220	101
3b	Tyr	242	0.53	274	0.53	0.219	100
3c	TOAC	230	0.53	274	0.53	0.170	78
4a	His	326	0.53	274	0.53	0.225	103
4b	Tyr	242	0.53	274	0.53	0.220	101

coupling	amino acid[a]			PyBOP		loading	yield
		[mg]	[mmol]	[mg]	[mmol]	[mmol/g]	[%]
4c	TOAC	230	0.53	274	0.53	0.116	53
5	Gly	470	1.58	822	1.58	0.235	108
6	*D*-Pro	533	1.58	822	1.58	0.216	99
7	Gly	470	1.58	822	1.58	0.224	103
8a	His	326	0.53	274	0.53	0.199	91
8b	Tyr	242	0.53	274	0.53	0.190	87
8c	TOAC	230	0.53	274	0.53	0.100	46
9	Gly	470	1.58	822	1.58	0.192	88
10a	His	326	0.53	274	0.53	0.192	88
10b	Tyr	242	0.53	274	0.53	0.211	97
10c	TOAC	230	0.53	274	0.53	0.125	57

[a] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.
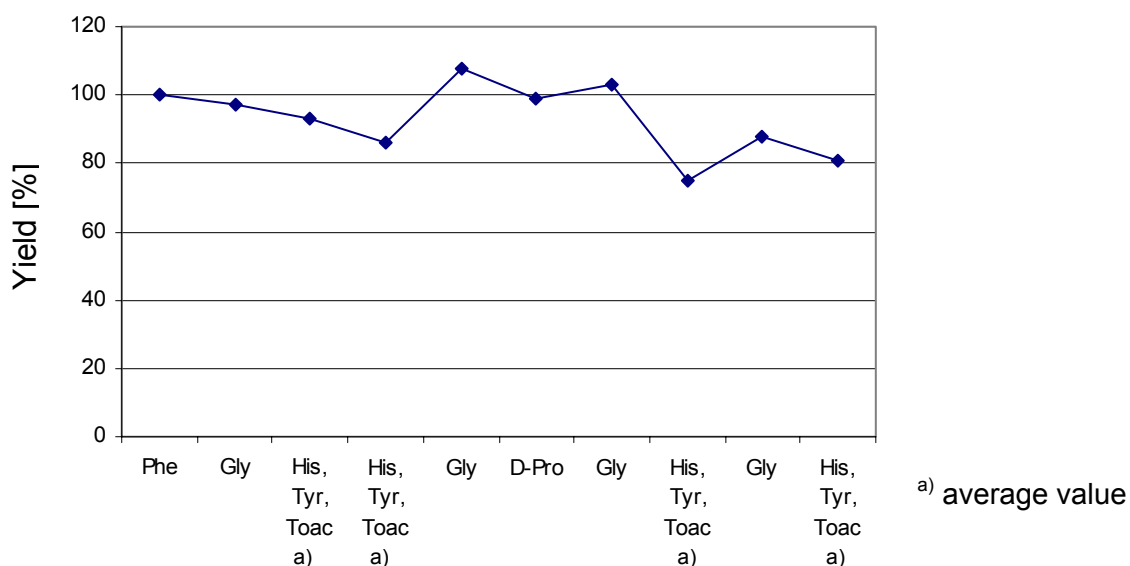
Figure 8-3: Coupling step yields determined by UV for the synthesis of the 81-member peptide library.

From the yields of each coupling step, the total yields for the 81 members of the library were calculated (**Table 8-8**).

Table 8-8: **Total yield for each peptide-member of the library.**

peptide-ligand	X_1	X_2	X_3	X_4	total yield [%]
96-01	Tyr	Tyr	Tyr	Tyr	80
96-02	Tyr	Tyr	Tyr	His	73
96-03	Tyr	Tyr	Tyr	TOAC	47
96-04	Tyr	Tyr	His	Tyr	84
96-05	Tyr	Tyr	His	His	76
96-06	Tyr	Tyr	His	TOAC	48
96-07	Tyr	Tyr	TOAC	Tyr	42
96-08	Tyr	Tyr	TOAC	His	38
96-09	Tyr	Tyr	TOAC	TOAC	25
96-10	Tyr	His	Tyr	Tyr	82
96-11	Tyr	His	Tyr	His	74
96-12	Tyr	His	Tyr	TOAC	48
96-13	Tyr	His	His	Tyr	85
96-14	Tyr	His	His	His	76
96-15	Tyr	His	His	TOAC	50
96-16	Tyr	His	TOAC	Tyr	44
96-17	Tyr	His	TOAC	His	40
96-18	Tyr	His	TOAC	TOAC	26
96-19	Tyr	TOAC	Tyr	Tyr	42
96-20	Tyr	TOAC	Tyr	His	38
96-21	Tyr	TOAC	Tyr	TOAC	25
96-22	Tyr	TOAC	His	Tyr	44
96-23	Tyr	TOAC	His	His	40
96-24	Tyr	TOAC	His	TOAC	26

peptide-ligand	X_1	X_2	X_3	X_4	total yield [%]
96-25	Tyr	TOAC	TOAC	Tyr	22
96-26	Tyr	TOAC	TOAC	His	20
96-27	Tyr	TOAC	TOAC	TOAC	13
96-28	His	Tyr	Tyr	Tyr	81
96-29	His	Tyr	Tyr	His	74
96-30	His	Tyr	Tyr	TOAC	47
96-31	His	Tyr	His	Tyr	85
96-32	His	Tyr	His	His	77
96-33	His	Tyr	His	TOAC	50
96-34	His	Tyr	TOAC	Tyr	43
96-35	His	Tyr	TOAC	His	39
96-36	His	Tyr	TOAC	TOAC	25
96-37	His	His	Tyr	Tyr	83
96-38	His	His	Tyr	His	75
96-39	His	His	Tyr	TOAC	48
96-40	His	His	His	Tyr	86
96-41	His	His	His	His	78
96-42	His	His	His	TOAC	51
96-43	His	His	TOAC	Tyr	44
96-44	His	His	TOAC	His	40
96-45	His	His	TOAC	TOAC	26
96-46	His	TOAC	Tyr	Tyr	43
96-47	His	TOAC	Tyr	His	39
96-48	His	TOAC	Tyr	TOAC	25
96-49	His	TOAC	His	Tyr	44

peptide-ligand	X$_1$	X$_2$	X$_3$	X$_4$	total yield [%]
96-50	His	TOAC	His	His	40
96-51	His	TOAC	His	TOAC	26
96-52	His	TOAC	TOAC	Tyr	23
96-53	His	TOAC	TOAC	His	21
96-54	His	TOAC	TOAC	TOAC	13
96-55	TOAC	Tyr	Tyr	Tyr	62
96-56	TOAC	Tyr	Tyr	His	57
96-57	TOAC	Tyr	Tyr	TOAC	37
96-58	TOAC	Tyr	His	Tyr	65
96-59	TOAC	Tyr	His	His	59
96-60	TOAC	Tyr	His	TOAC	38
96-61	TOAC	Tyr	TOAC	Tyr	33
96-62	TOAC	Tyr	TOAC	His	30
96-63	TOAC	Tyr	TOAC	TOAC	19
96-64	TOAC	His	Tyr	Tyr	64
96-65	TOAC	His	Tyr	His	58
96-66	TOAC	His	Tyr	TOAC	37
96-67	TOAC	His	His	Tyr	67
96-68	TOAC	His	His	His	60
96-69	TOAC	His	His	TOAC	39
96-70	TOAC	His	TOAC	Tyr	34
96-71	TOAC	His	TOAC	His	31
96-72	TOAC	His	TOAC	TOAC	20
96-73	TOAC	TOAC	Tyr	Tyr	33
96-74	TOAC	TOAC	Tyr	His	30

peptide-ligand	X_1	X_2	X_3	X_4	total yield [%]
96-75	TOAC	TOAC	Tyr	TOAC	19
96-76	TOAC	TOAC	His	Tyr	34
96-77	TOAC	TOAC	His	His	31
96-78	TOAC	TOAC	His	TOAC	20
96-79	TOAC	TOAC	TOAC	Tyr	17
96-80	TOAC	TOAC	TOAC	His	16
96-81	TOAC	TOAC	TOAC	TOAC	11

8.7.3 Acetylation of the *N*-terminus of the decapeptides

IV-MAG-6

X_n = His(Trt), Tyr(*t*-Bu), TOAC

96

X_n = His(Trt), Tyr(*t*-Bu), TOAC

97

1) Ac$_2$O, 3 % NMM in DMF

96 $\longrightarrow$ **97**

2) test for free amino functions
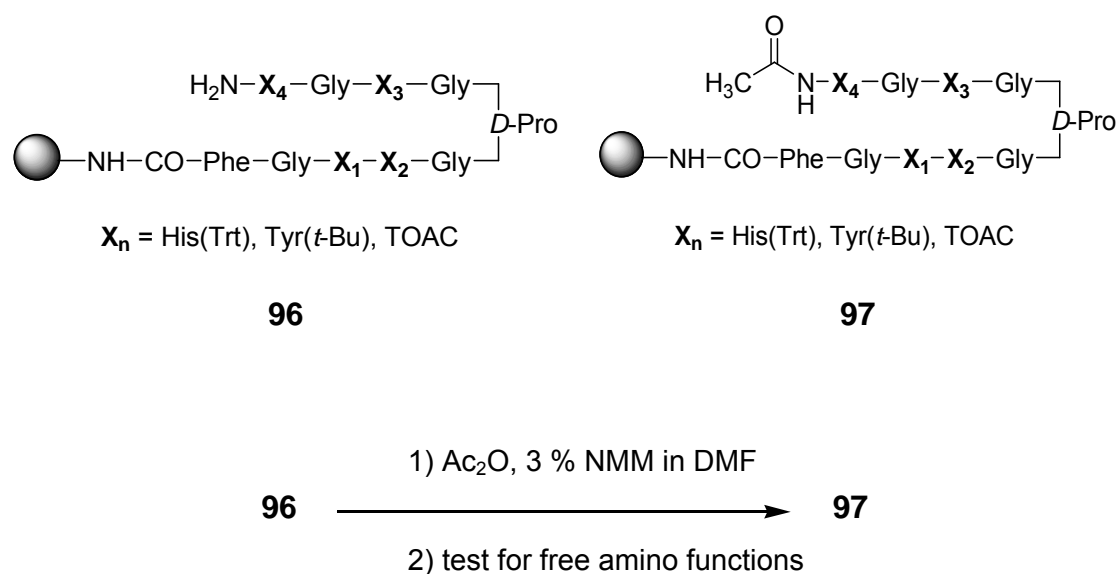
The 81 MicroKans™ containing the library of decapeptides (0.63 mmol of peptides) were added to 100 mL of a 3 % NMM solution in absolute DMF. 150 µL (1.58 mmol, 2.50 eq) of acetic anhydride were added to this solution and the reaction mixture was shaken at room temperature under argon for 12 hours. The reaction mixture was filtered and the resin washed 3 times with DMF. A few beads of **97** were withdrawn from a MikroKan™ and a NF31 test was performed in order to check the completion of the reaction. The colour test was negative, indicating that the reaction had been

successful. By comparison, the test performed on the starting material **96** was positive.

8.7.4   Cleavage of the side chain protecting groups

IV-MAG-8
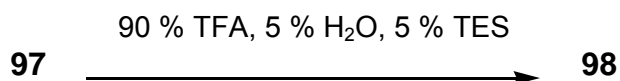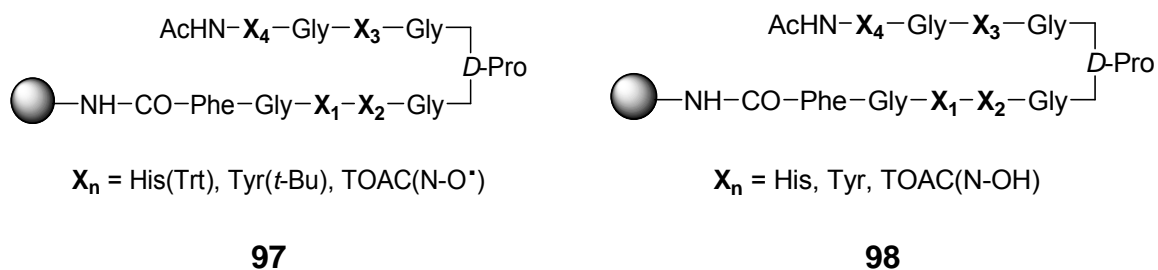
The 81 MikroKans™ containing 0.63 mmol of peptides were washed 3 times with dichloromethane, then 3 times with methanol in order to remove the DMF. The peptides were shaken in a mixture of 90 % TFA, 5 % water and 5 % triethylsilane at room temperature for 5 hours. The solution was filtered and the procedure was repeated to ensure complete deprotection of the side chains. The yellow solution was filtered and the resin was washed successively with dichloromethane, methanol and water.

8.7.5 Regeneration of the nitroxyl radical of the TOAC moieties

IV-MAG-9

AcHN−X_4−Gly−X_3−Gly⌐
                               D-Pro
⬤—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_n = His, Tyr, TOAC(N-OH)

98

AcHN−X_4−Gly−X_3−Gly⌐
                               D-Pro
⬤—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_n = His, Tyr, TOAC(N-O•)

99

10 % aq. NH_3, air

98 $\longrightarrow$ **99**
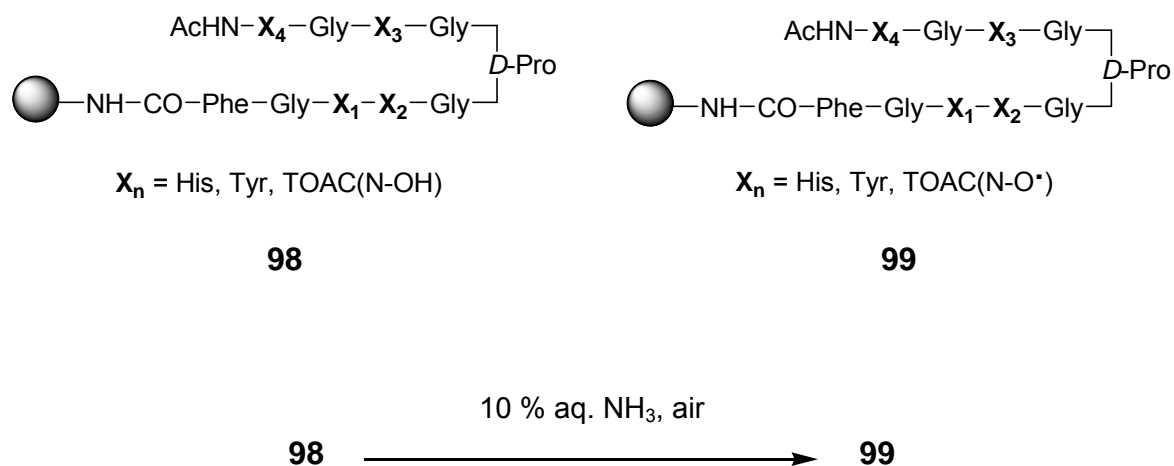
The 81 MikroKans™ containing 0.63 mmol of decapeptides were shaken 4 hours in 10 % aqueous ammonia. The solution was filtered and the procedure was repeated a second time. The library of peptides was washed successively with water and methanol. Finally, the resin carrying the peptide–ligands was dried under reduced pressure.

8.7.6 Complexation of the peptide–ligands with copper(II)

IV-MAG-49
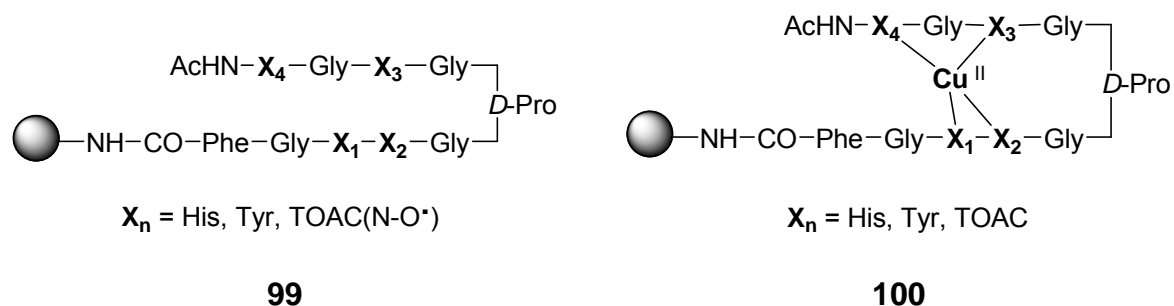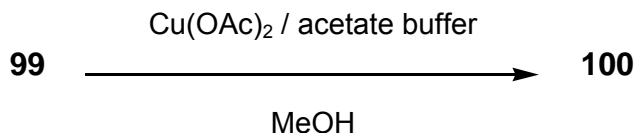
AcHN−X_4−Gly−X_3−Gly⌐
                               D-Pro
⬤—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_n = His, Tyr, TOAC(N-O•)

99

AcHN−X_4−Gly−X_3−Gly⌐
              Cu^{II} D-Pro
⬤—NH−CO−Phe−Gly−X_1−X_2−Gly⌐

X_n = His, Tyr, TOAC

100

$$99 \xrightarrow[\text{MeOH}]{\text{Cu(OAc)}_2 \text{ / acetate buffer}} 100$$

10.0 mg (2.00 μmol, 1.00 eq) of the solid-supported peptide–ligands were washed twice with methanol. Then 2 mL of methanol, 350 μg (2.00 μmol, 1.00 eq) of copper(II) acetate, 11.0 μL of acetic acid and 20.0 mg of sodium acetate (100 mM acetate buffer) were added to the resin. The reaction mixture was shaken at room temperature under argon for 5 hours and the resin was washed 3 times with methanol. The colour of the beads before and after complexation was compared. While the peptide-ligands had a white to pale yellow colour, the complexes gave a range of colours from yellow–orange to green–blue according to the structure of the peptide. Peptides containing histidine residues tend to give blue-coloured complexes. Peptides containing tyrosine residues tend to give green-coloured complexes. Peptides containing TOAC residues tend to give orange-coloured complexes.

8.8 Screening of the TOAC library, 99

8.8.1 Catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39 with copper(II) complexes of peptide–ligands 99

IV-MAG-13
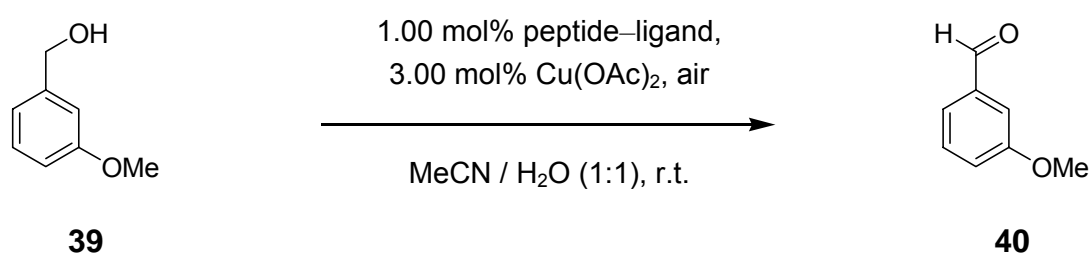
0.73 mg (4.00 μmol, 1.00 mol%) of copper(II) acetate were dissolved in 2.00 mL of a 1:1 mixture of acetonitrile and water in a 5 mL vial. 20.0 mg (4.00 μmol, 1.00 mol%) of solid-supported peptide–ligand from the TOAC library **99** were added, followed by 50.0 μL (400 μmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39**. The resulting mixture was shaken at room temperature with the vial open to air.

The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 µL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at λ = 310 nm.

As no evolution of the absorbance was observed after 24 hours, 1.45 mg (8.00 µmol, 2.00 mol%) of copper(II) acetate were added to each vial.

The quantity of 3-methoxybenzaldehyde formed after 1.5, 2.5, 3.5 and 24 hours is summarised in **Table 8-9**. The time t = 0 corresponds to the time when the additional copper(II) acetate was added.

For some of the members of the library, the catalysis was repeated (adding diphenylether as the internal standard) and the reaction mixture was analysed by GC after 24 hours. These results appear in brackets in **Table 8-9**.

Table 8-9: **Screening of the TOAC library 99 for catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39.**

peptide– ligand	peptide sequence[a]				µmol of 3-methoxybenzaldehyde after:			
	X_1	X_2	X_3	X_4	1.5 h	2.5 h	3.5 h	24 h[b]
99-01	Y	Y	Y	Y	0.49	0.55	0.63	0.66
99-02	Y	Y	Y	H	0.21	0.17	0.15	0.34
99-03	Y	Y	Y	T'	0.15	0.56	0.70	4.94 (4.03)
99-04	Y	Y	H	Y	0.95	1.13	1.17	1.40
99-05	Y	Y	H	H	0.54	0.51	0.62	0.76
99-06	Y	Y	H	T'	0.26	0.56	0.77	3.36
99-07	Y	Y	T'	Y	0.39	0.91	1.16	4.96 (4.56)
99-08	Y	Y	T'	H	1.71	2.55	3.08	3.95 (4.02)
99-09	Y	Y	T'	T'	0.51	1.27	2.02	21.7 (18.5)
99-10	Y	H	Y	Y	0.05	0.01	0.05	0.00
99-11	Y	H	Y	H	0.00	0.07	0.09	0.09

peptide–ligand	peptide sequence[a]				μmol of 3-methoxybenzaldehyde after:			
	X_1	X_2	X_3	X_4	1.5 h	2.5 h	3.5 h	24 h[b]
99-12	Y	H	Y	T'	0.21	0.52	0.83	4.27
99-13	Y	H	H	Y	0.16	0.28	0.31	0.00
99-14	Y	H	H	H	0.06	0.13	0.14	0.00
99-15	Y	H	H	T'	0.36	0.69	1.07	4.09
99-16	Y	H	T'	Y	0.40	0.75	1.05	11.5 (11.9)
99-17	Y	H	T'	H	0.52	1.07	1.51	6.12
99-18	Y	H	T'	T'	0.65	1.67	2.64	15.1 (17.4)
99-19	Y	T'	Y	Y	0.24	0.61	1.01	6.57 (8.04)
99-20	Y	T'	Y	H	0.31	0.67	1.03	4.08
99-21	Y	T'	Y	T'	0.61	1.28	1.76	11.2 (13.6)
99-22	Y	T'	H	Y	0.31	0.83	1.32	6.77
99-23	Y	T'	H	H	0.28	0.51	0.81	5.15
99-24	Y	T'	H	T'	0.76	1.17	1.40	6.64
99-25	Y	T'	T'	Y	0.43	0.83	1.31	5.14
99-26	Y	T'	T'	H	0.66	1.13	1.33	7.17
99-27	Y	T'	T'	T'	0.96	1.77	2.61	13.1 (11.4)
99-28	H	Y	Y	Y	0.18	0.00	0.03	0.00
99-29	H	Y	Y	H	0.00	0.00	0.00	0.16
99-30	H	Y	Y	T'	0.22	0.29	0.69	3.49
99-31	H	Y	H	Y	0.03	0.00	0.00	0.09
99-32	H	Y	H	H	0.47	0.46	0.47	0.59
99-33	H	Y	H	T'	0.33	0.62	0.98	4.87
99-34	H	Y	T'	Y	0.30	0.50	0.80	3.45
99-35	H	Y	T'	H	0.25	0.41	0.70	3.58

peptide–ligand	peptide sequence[a]				μmol of 3-methoxybenzaldehyde after:			
	X_1	X_2	X_3	X_4	1.5 h	2.5 h	3.5 h	24 h[b]
99-36	H	Y	T'	T'	0.57	0.97	1.58	7.53 (5.91)
99-37	H	H	Y	Y	0.00	0.00	0.01	0.30
99-38	H	H	Y	H	0.01	0.03	0.11	0.05
99-39	H	H	Y	T'	0.16	0.29	0.50	3.57
99-40	H	H	H	Y	0.00	0.00	0.00	0.10
99-41	H	H	H	H	0.32	0.31	0.32	0.51
99-42	H	H	H	T'	0.14	0.28	0.44	3.16 (3.02)
99-43	H	H	T'	Y	0.13	0.29	0.36	2.24
99-44	H	H	T'	H	0.15	0.84	0.57	3.52
99-45	H	H	T'	T'	0.47	0.99	1.42	8.07 (9.98)
99-46	H	T'	Y	Y	0.17	0.31	0.43	2.67
99-47	H	T'	Y	H	0.10	0.37	0.50	2.30
99-48	H	T'	Y	T'	0.52	1.07	1.39	6.74 (8.12)
99-49	H	T'	H	Y	0.20	0.37	0.55	3.29
99-50	H	T'	H	H	0.16	0.42	0.51	3.61
99-51	H	T'	H	T'	0.46	0.95	1.56	6.85 (6.98)
99-52	H	T'	T'	Y	1.02	1.65	2.34	8.77 (10.4)
99-53	H	T'	T'	H	0.33	1.02	1.34	7.32 (7.05)
99-54	H	T'	T'	T'	0.84	1.61	2.11	7.63 (8.09)
99-55	T'	Y	Y	Y	0.15	0.34	0.38	2.04
99-56	T'	Y	Y	H	0.23	0.50	0.73	3.30
99-57	T'	Y	Y	T'	0.22	0.42	0.76	3.65
99-58	T'	Y	H	Y	0.25	0.42	0.46	2.26
99-59	T'	Y	H	H	0.23	0.48	0.72	3.31

peptide–ligand	peptide sequence[a]				μmol of 3-methoxybenzaldehyde after:			
	X_1	X_2	X_3	X_4	1.5 h	2.5 h	3.5 h	24 h[b]
99-60	T'	Y	H	T'	0.64	1.01	1.48	5.70
99-61	T'	Y	T'	Y	0.66	1.10	1.51	4.35 (3.23)
99-62	T'	Y	T'	H	0.72	1.31	1.77	9.39
99-63	T'	Y	T'	T'	2.06	3.12	4.34	17.5 (19.6)
99-64	T'	H	Y	Y	0.39	0.62	0.84	4.19
99-65	T'	H	Y	H	0.58	1.01	1.24	5.24
99-66	T'	H	Y	T'	0.82	1.42	1.84	6.95
99-67	T'	H	H	Y	0.38	0.59	0.97	4.88
99-68	T'	H	H	H	0.24	1.12	0.59	3.65
99-69	T'	H	H	T'	0.58	1.61	2.12	8.40
99-70	T'	H	T'	Y	0.62	1.52	1.92	11.1 (11.3)
99-71	T'	H	T'	H	0.63	1.03	1.46	7.29
99-72	T'	H	T'	T'	1.72	3.03	3.85	14.7 (15.3)
99-73	T'	T'	Y	Y	0.42	0.95	1.08	4.75
99-74	T'	T'	Y	H	0.88	1.09	1.74	6.74
99-75	T'	T'	Y	T'	1.19	2.85	3.42	15.6 (12.7)
99-76	T'	T'	H	Y	1.01	0.92	1.18	5.85
99-77	T'	T'	H	H	0.30	0.55	0.74	4.12
99-78	T'	T'	H	T'	1.46	1.89	2.89	10.5 (11.6)
99-79	T'	T'	T'	Y	0.70	1.37	1.69	6.99
99-80	T'	T'	T'	H	1.14	2.27	2.86	10.1 (9.67)
99-81	T'	T'	T'	T'	0.53	1.77	1.99	7.64

[a] Y = tyrosine, H = histidine, T' = TOAC

[b] The values in bracket correspond to the results obtained by GC analysis.

8.8.2 Control experiments for the catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39

8.8.2.1 Effect of copper on the catalytic aerobic oxidation of 3-methoxybenzyl alcohol **39**

V-MAG-10
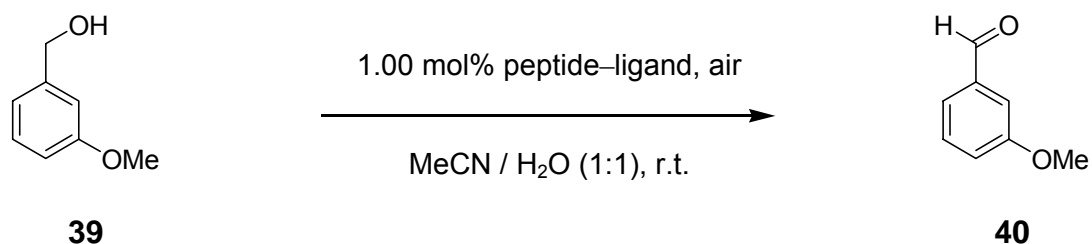
39 → **40**

A 5 mL vial was charged with 20.0 mg (4.00 µmol, 1.00 mol%) of solid-supported peptide–ligand from the TOAC library (**99-75**, **99-78** or **99-81**). 2.00 mL of a 1:1 mixture of acetonitrile and water was then added, followed by 50.0 µL (400 µmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39**. The resulting mixture was shaken at room temperature with the vial open to air. The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 µL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at $\lambda = 310$ nm. The quantity of 3-methoxybenzaldehyde **40** formed after 1.5, 2.5 and 24 hours is summarised in **Table 8-10**.

Table 8-10: Comparison of 3 peptide–ligands with or without copper salt in the catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39.

peptide–ligand	Cu(OAc)$_2$ [mol%]	μmol of 3-methoxybenzaldehyde after:		
		1.5 h	2.5 h	24 h
99-75	3.00	1.19	2.85	15.6
99-75	-	-	-	0.60
99-78	3.00	1.46	1.89	10.5
99-78	-	-	-	0.50
99-81	3.00	0.53	1.77	7.64
99-81	-	-	-	0.50

8.8.2.2 Catalytic effect of non-peptidic TOAC and *L*-tyrosine

IV-MAG-16
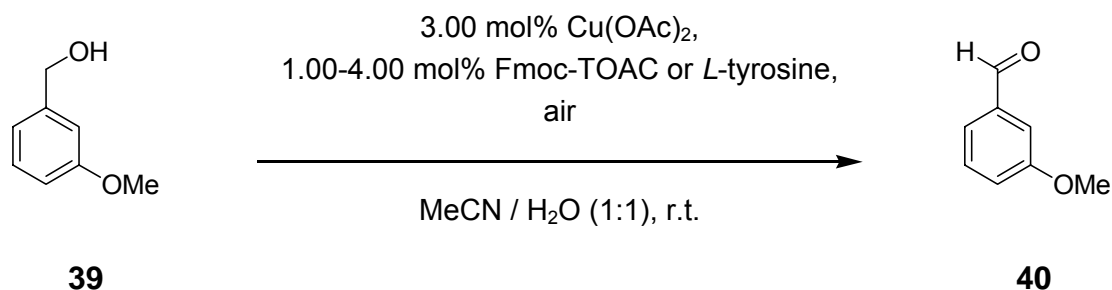
In a 5 mL vial, 2.18 mg (12.0 µmol, 3.00 mol%) of copper(II) acetate were dissolved in 2.00 mL of a 1:1 mixture of acetonitrile and water. 1.00, 2.00, 3.00 or 4.00 mol% of Fmoc-TOAC-OH and/or 1.00, 2.00, 3.00 or 4.00 mol% of H$_2$N-*L*-Tyr-OH were added as given in **Table 8-11**. 50.0 µL (400 µmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39** were added and the resulting mixture was shaken at room temperature with the vial open to air. The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 µL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at λ = 310 nm.

Table 8-11: Control experiments for the catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39: catalytic effect of non-peptidic TOAC 43 and *L*-tyrosine.

entry	Cu(OAc)$_2$		Fmoc-TOAC, **43**		L-tyrosine		3-methoxybenzaldehyde [μmol]		
	[mg]	[mol%]	[mg]	[mol%]	[mg]	[mol%]	2.5 h	3.5 h	24 h
1	-	-	-	-	-	-	0.09	0.00	0.25
2	2.18	3.00	-	-	-	-	0.07	0.00	0.57
3	-	-	1.70	1.00	-	-	0.00	0.00	0.43
4	-	-	3.50	2.00	-	-	0.00	0.00	0.38
5	-	-	5.20	3.00	-	-	0.00	0.00	0.42
6	-	-	7.00	4.00	-	-	0.00	0.16	1.45
7	2.18	3.00	1.70	1.00	-	-	1.17	1.78	4.23
8	2.18	3.00	3.50	2.00	-	-	1.36	1.95	4.32
9	2.18	3.00	5.20	3.00	-	-	1.57	2.34	4.25
10	2.18	3.00	7.00	4.00	-	-	1.59	2.01	5.05
11	-	-	-	-	0.70	1.00	0.08	0.18	0.94
12	-	-	-	-	1.40	2.00	0.08	0.20	0.89
13	-	-	-	-	2.20	3.00	0.07	0.36	1.05
14	-	-	-	-	2.90	4.00	0.12	0.03	0.43
15	2.18	3.00	-	-	0.70	1.00	0.03	0.00	0.25
16	2.18	3.00	-	-	1.40	2.00	0.00	0.00	2.10
17	2.18	3.00	-	-	2.20	3.00	0.00	0.09	0.63
18	2.18	3.00	-	-	2.90	4.00	0.00	0.62	1.82
19	2.18	3.00	3.50	2.00	1.40	2.00	0.37	0.00	0.25

8.8.2.3 Catalysis under inert atmosphere

V-MAG-10

39 → **40**
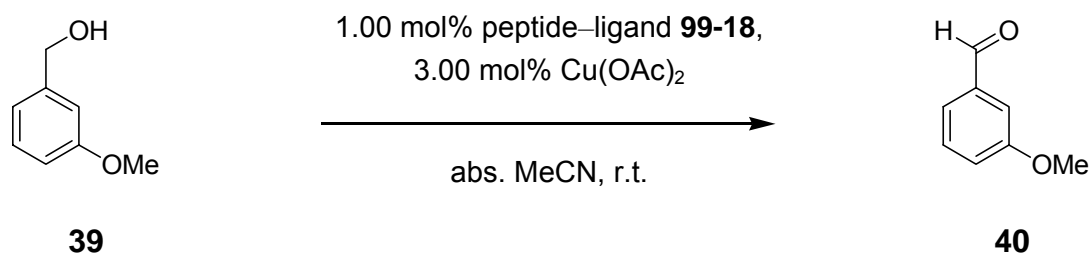
In a 5 mL *Schlenk* flask equipped with a septum and a gas inlet, 2.18 mg (12.0 µmol, 3.00 mol%) of copper(II) acetate were dissolved in 2.00 mL of absolute acetonitrile. 20.0 mg (4.00 mmol, 1.00 mol%) of the solid-supported peptide-ligand from the TOAC library (**99-18**) were added. The flask was evacuated and filled with argon. 50.0 µL (400 µmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39** were added and the resulting mixture was shaken at room temperature under argon. The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 µL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at $\lambda = 310$ nm. Only 0.25 µmol of 3-methoxybenzaldehyde were formed after 24 hours.

8.8.3   Recycling of the peptide–copper catalysts

V-MAG-11

39 → **40**
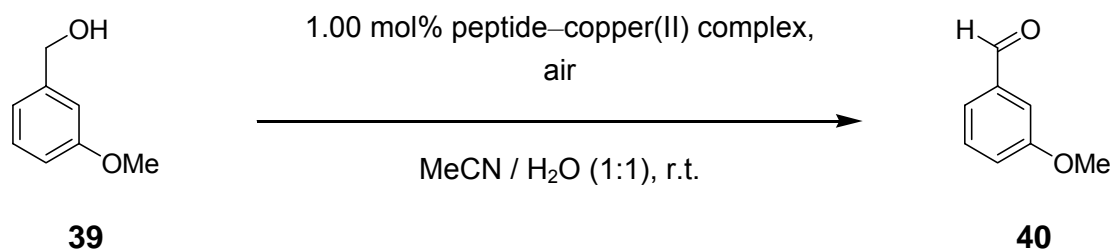
Three of the peptide–copper(II) complexes from the library **99** (**99-63**, **99-71** and **99-78**) used for the catalytic aerobic oxidation of 3-methoxybenzyl alcohol **39** were

washed with water and dried under reduced pressure. A 5 mL vial was charged with 20.0 mg (4.00 µmol, 1.00 mol%) of solid-supported peptide–copper(II) complex **99-63**, **99-71** or **99-78**, 2.00 mL of a 1:1 mixture of acetonitrile and water, followed by 50.0 µL (400 µmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39**. Eventually, 1.30 mg (7.33 µmol, 2.00 mol%) of copper(II) acetate were added (**Table 8-12**) and the resulting mixture was shaken at room temperature with the vial open to air. The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 μL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at λ = 310 nm. The quantity of 3-methoxybenzaldehyde **40** formed after 1.5, 2.5 and 24 hours is summarised in **Table 8-12**.

Table 8-12: Quantity of 3-methoxybenzaldehyde 40 formed after 1.5, 2.5 and 24 hours for the second use of the catalysts.

peptide–copper(II) complex	Cu(OAc)$_2$ [mol %]	μmol of 3-methoxybenzaldehyde **40** after:		
		1.5 h	2.5 h	24 h
99-63	-	0.1	0.3	1.3
99-71	-	0.1	0.4	1.5
99-78	-	0.1	0.2	1.3
99-43	2.00	0.1	0.2	1.0
99-44	2.00	0.4	0.3	1.6
99-80	2.00	0.3	0.7	2.0

8.9 Synthesis of one of the members of the library on TentaGel *Wang* resin and catalysis with the soluble peptide–ligand

8.9.1 Synthesis of the decapeptide 103 following the Fmoc strategy

V-MAG-6
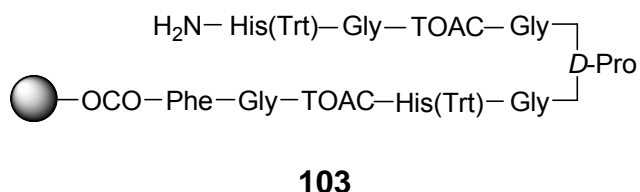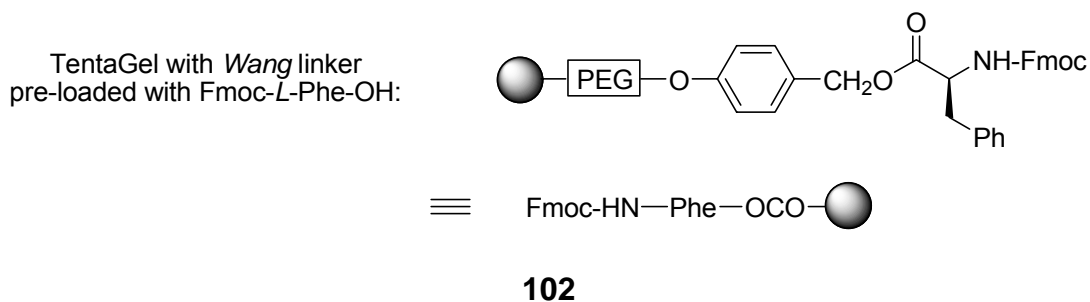
102

103

1) 20 % piperidine in DMF

2) 2.50 eq Fmoc-Gly-OH, 2.50 eq PyBOP, 3 % NMM in DMF

3) test for free amino functions

4) 20 % piperidine in DMF

102 ⟶ **103**

5) repetition of the steps 2) to 4) with the Fmoc-protected amino acids, Fmoc-TOAC-OH **43**, Fmoc-*L*-His(Trt)-OH, Fmoc-Gly-OH or Fmoc-*D*-Pro-OH, according to the given decapeptide sequence

A 100 mL *Merrifield* flask was charged with 1.50 g (theoretical loading = 0.23 mmol/g) of TentaGel *Wang* resin pre-loaded with Fmoc-*L*-Phe-OH. 15 mL of absolute DMF were added and the resin was allowed to swell by gentle shaking at room

temperature for 1 hour. The Fmoc protecting groups were cleaved according to the general procedure (8.2.6) and the loading of the resin was determined to be 0.24 mmol/g. 15 mL of a 3 % NMM solution in absolute DMF were added to the resin, followed by 2.50 eq of Fmoc-protected amino acid (Fmoc-Gly-OH, Fmoc-TOAC-OH **43**, Fmoc-*L*-Tyr(Trt)-OH or Fmoc-*D*-Pro-OH) and 449 mg (0.86 mmol, 2.50 eq) of the coupling agent PyBOP. The resulting suspension was shaken at room temperature under argon for 12 hours. The solution was filtered and the resin washed 3 times with 15 mL of DMF. The completion of the reaction was checked with the NF31 test. If necessary the coupling step was repeated. **Table 8-13** summarises the number of times the coupling was repeated and the yield obtained for each step. Once the colour test was negative, the Fmoc protecting group was removed and the coupling was quantified by UV.

Table 8-13: Synthesis of the decapeptide 103 on TentaGel *Wang* resin. Quantities of amino acid used, number of coupling steps and yield for each coupling.

amino acid[a]			Nr of couplings	loading	yield
	[mg]	[mmol]		[mmol/g]	[%]
Phe	-	-	-	0.239	100
Gly	256	0.86	1	0.249	104
TOAC	377	0.86	1	0.143	60
His	534	0.86	3	0.234	98
Gly	256	0.86	1	0.246	103
D-Pro	291	0.86	1	0.246	103
Gly	256	0.86	3	0.229	96
TOAC	377	0.86	1	0.129	54
Gly	256	0.86	3	0.170	71
His	534	0.86	3	0.172	72

[a] All amino acids are in the *L*-configuration, except for proline which has the *D*-configuration.

8.9.2 Acetylation of the *N*-terminus of the decapeptide 103

V-MAG-6

103   **104**

$$\textbf{103} \xrightarrow[\text{2) test for free amino functions}]{\text{1) Ac}_2\text{O, 3 \% NMM in DMF}} \textbf{104}$$
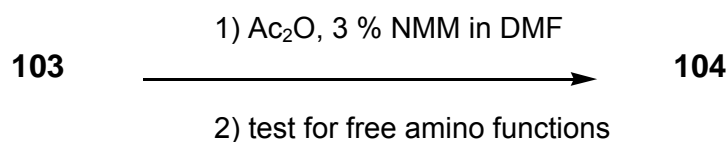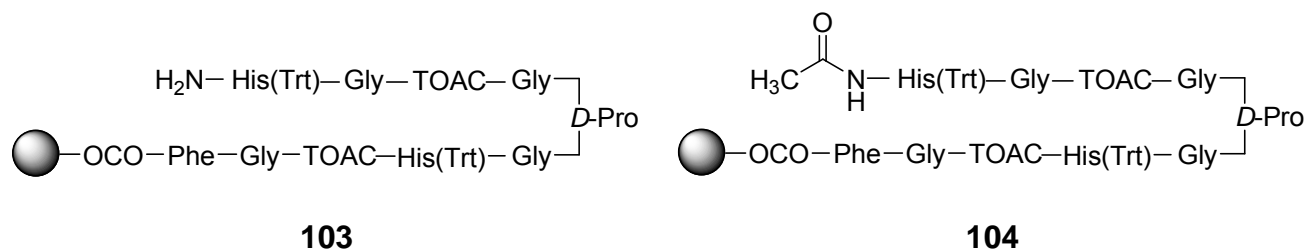
15 mL of a 3 % NMM solution in absolute DMF were added to the resin. 82.0 μL (860 μmol, 2.50 eq) of acetic anhydride were added to the suspension and the reaction mixture was shaken at room temperature under argon for 12 hours. The solution was filtered and the resin washed 3 times with DMF. A few beads were taken and a NF31 test was performed in order to check the completion of the reaction. The colour test was negative, indicating that the reaction had been successful. By comparison, the test performed on the starting material **103** was positive.

8.9.3 Deprotection of the side chains and cleavage from the TentaGel *Wang* resin

V-MAG-6

104   **101**

90 % TFA, 5 % H$_2$O, 5 % TIS

104 $\xrightarrow{\hspace{4cm}}$ **101**

The resin was washed 3 times with dichloromethane and 3 times with methanol in order to remove the DMF. It was shaken 5 hours in 15 mL of a mixture of 90 % of TFA, 5 % of water and 5 % of triisopropylsilane. The yellow solution was filtered and the filtrate was concentrated under reduced pressure. The product was precipitated by the addition of diethylether. The product was filtered, dissolved in 80 mL of a mixture of water and methanol (90 : 10) and lyophilised. 382 mg of crude product were obtained. 50 mg were purified by preparative HPLC (RP-18, water : acetonitrile : acetic acid = 90 : 10 : 0.5) to give 38 mg of pure peptide. The analysis by LC–MS showed that the obtained peptide had two hydroxyl amine moieties (**105**).

105

C$_{56}$H$_{82}$N$_{16}$O$_{14}$ 1203.35 g·mol^{-1}

Yield   290 mg, 0.24 mmol, 64 %

HPLC τ_R = 9.6 mins   (RP-18,   water : acetonitrile : acetic   acid   =
                  90 : 10 : 0.5)

MS   (positive ESI, H_2O) m/z (%, formula) = 1203 ([M]$^+$), 1204 ([M+H]$^+$),
                  1125 ([M+Na]$^+$), 1226 ([M+Na+H]$^+$).

8.9.4 Catalysis with the soluble peptide-ligand 105

V-MAG-12
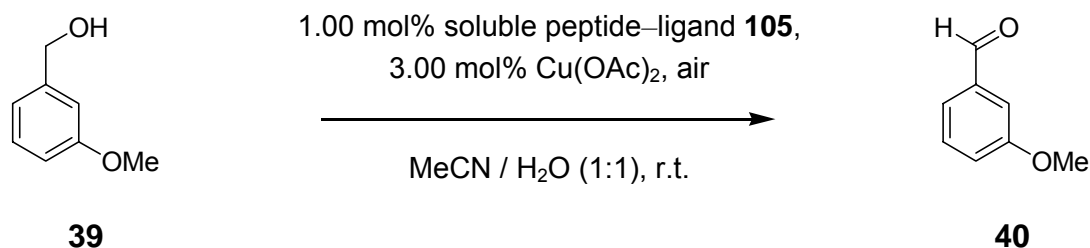
In a 5 mL vial, 2.18 mg (12.0 mmol, 3.00 mol%) of copper(II) acetate were dissolved in 2.00 mL of a 1:1 mixture of acetonitrile and water. 4.8 mg (4.00 µmol, 1.00 mol%) of the soluble peptide–ligand **101** were added, followed by 50.0 µL (400 µmol, 200 mM, 1.00 eq) of the test substrate 3-methoxybenzyl alcohol **39**. The resulting mixture was shaken at room temperature with the vial open to air. The reaction was followed by UV. For this purpose, aliquots of 50.0 µL were withdrawn from the reaction mixture and added to 500 µL of acetonitrile / water (1:1). The resulting solution was filtered through cotton wool and 50.0 µL of the filtrate were transferred into a 384-well titerplate for analysis at λ = 310 nm. After 24 hours, 14.32 µmol of 3-methoxybenzaldehyde **39** were formed, which corresponds to a yield in aldehyde of 3.6 %.

8.10 Synthesis of the resin-supported low molecular weight ligand 66

8.10.1 Synthesis of the resin-supported tosylamide 67[117]

I-MAG-32

69   **67**

= 1 % cross-linked polystyrene resin
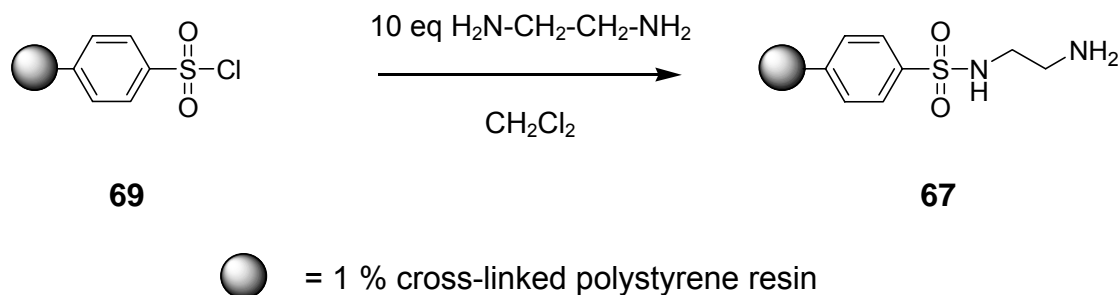
A 100 mL *Merrifield* flask was charged with 100 mg (144 μmol, 1.00 eq) of chlorosulfonated polystyrene resin PS-TsCl (loading = 1.44 mmol/g) **69** and 10 mL of dry dichloromethane. The resin was allowed to swell by gentle shaking at room temperature for 1 hour. The solvent was filtered and a solution of 87.0 mg (1.44 mmol, 10.0 eq) of ethylenediamine in 10 mL of dry dichloromethane was added. The suspension was shaken at room temperature under argon overnight. The solvent was filtered and the resin was washed 3 times with dichloromethane. To check the completion of the reaction, a NF31 test was performed on a few beads of product **67**. The red colour of the beads indicated the presence of free amino functions. When the same colour test was performed on the starting material, the beads remained colourless.

8.10.2 Synthesis of the resin-supported tosylamide ligand 66[118]

II-MAG-33

68[a][89]

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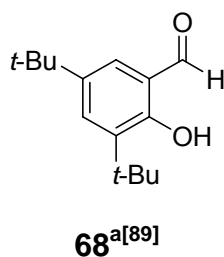
[a] 2,4-di-*tert*-butyl-salicylaldehyde **68** was synthesised by Stephan Bulat.

67   **66**
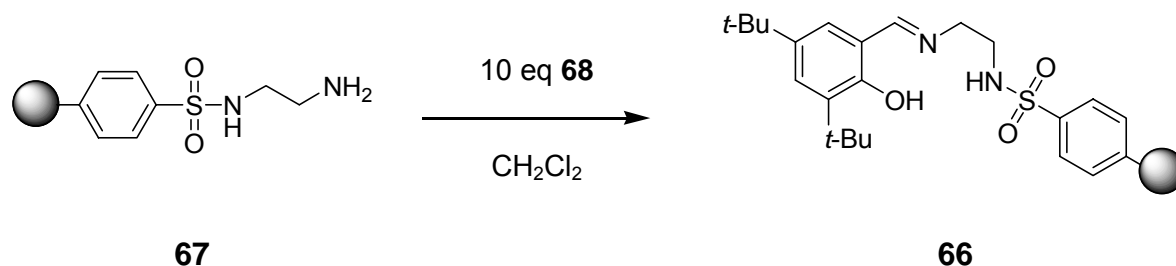
10 mL of dichloromethane and 337 mg (1.44 mmol, 10.0 eq) of 2,4-di-*tert*-butyl-salicylaldehyde **68** were added to 100 mg (144 μmol, 1.00 eq) of resin **67** (loading = 1.44 mmol/g). The solution was shaken at room temperature under argon for 24 hours. The solvent was removed by filtration, the resin was washed 3 times with dichloromethane and dried under reduced pressure to give ligand **66**.

66
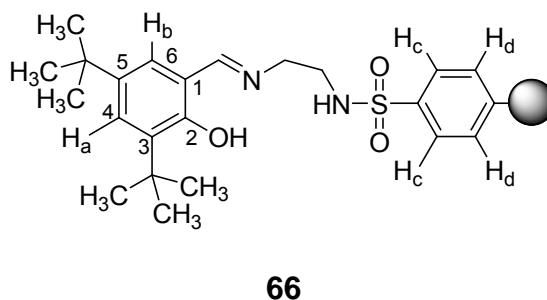
FT-IR	(KBr disc) $\tilde{v}$ [cm^{-1}] = 3298 [br, ν(O–H) + ν(N–H)], 1627 [s, ν(C=N)], 1158 [s, ν(C–O)].
HR-MAS ^{1}**H-NMR**	(300 MHz, CDCl$_3$) δ = 1.40 (s, 9H, *t*-Bu), 1.55 (s, 9H, *t*-Bu), 3.25 (s, 2H, CH$_2$), 3.70 (s, 2H, CH$_2$), 5.20-5.30 (s, 1H, NH), 6.61 (s, 1H, H$_a$), 6.73 (s, 1H, H$_d$), 7.19 (s, 1H, H$_b$), 7.55 (s, 1H, H$_c$), 8.39 (s, 1H, OH), 11.3-11.6 (m, 1H, N=CH).

HR-MAS	(75 MHz, CDCl$_3$) δ = 29.9 (q, CH$_3$ / *t*-Bu), 31.2 (q, CH$_3$ / *t*-Bu), 34.9
^{13}C-NMR	(s, C$_q$ / *t*-Bu), 36.1 (s, C$_q$ / *t*-Bu), 40.8 (d, CH$_2$–NHTos), 60.0 (d,
	CH$_2$–N=), 119.9 (s, aryl–C), 128.5 (d, 4 aryl–C from Tos), 138.7 (s,
	aryl–C–S), 141.2 (s, aryl–C), 144.8 (s, aryl–C), 159.3 (s, aryl–C$_2$),
	169.1 (s, C=N).

8.10.3 Complexation of the resin-supported tosylamide ligand 66 with copper[90]

I-MAG-36

66   **65**

Cu(OAc)$_2$, KOH

66 ⟶ **65**

MeCN, MeOH
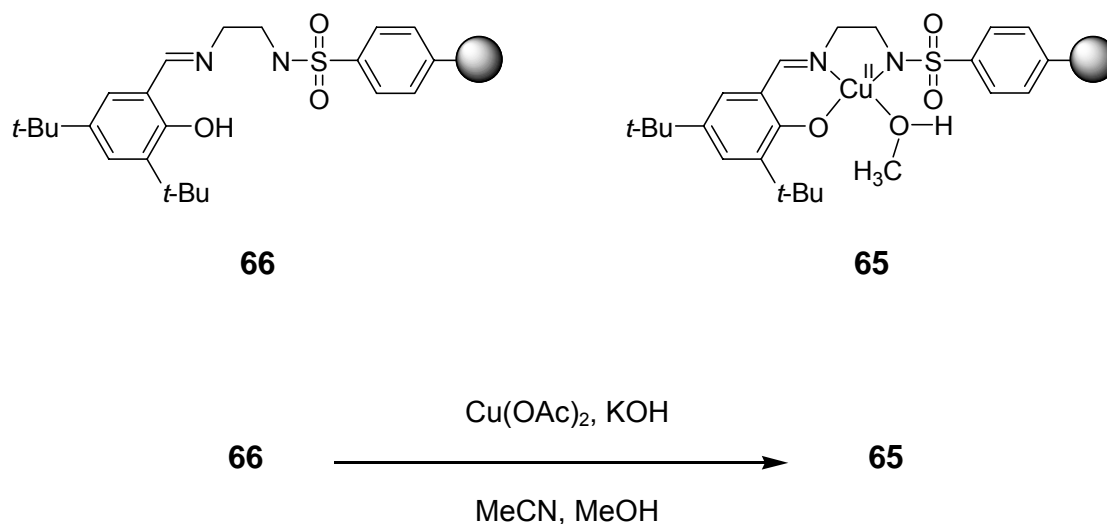
To 100 mg (144 μmol, 1.00 eq) of resin **66** (loading = 1.44 mmol/g), 10 mL of acetonitrile, 24.0 mg (430 μmol, 3.00 eq) of potassium hydroxide and 26.0 mg (144 μmol, 1.00 eq) of copper(II) acetate were added. The suspension was shaken at room temperature under argon. After 3 hours, 10 mL of methanol were added and the reaction mixture was shaken further overnight. The solution, green at the beginning, turned yellow overnight. The solvent was filtered and the resin was washed several times with methanol until a clean green resin was obtained.

8.11 Catalytic aerobic oxidation of primary alcohols with different low molecular weight ligands

8.11.1 Comparison of the soluble sulfonamide ligand 31 and its resin-supported equivalent 66[89]

66

31[89]
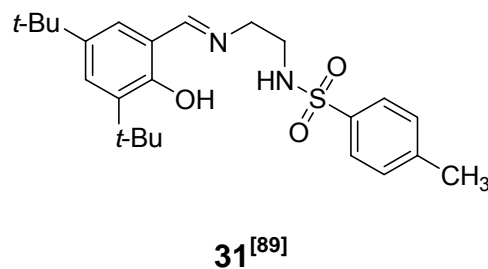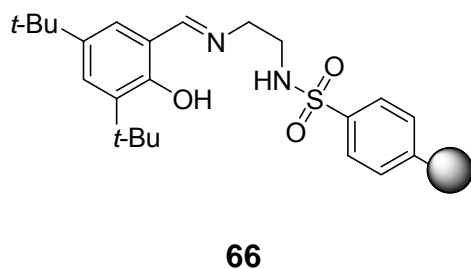
8.11.1.1 Aerobic oxidation of benzyl alcohol **33** to benzaldehyde **34**

III-MAG-17

0.50 mol% ligand **66** or **31**,
0.50 mol% Cu salt, 5.00 mol% KOH,
air

MeCN, r.t.

33

34
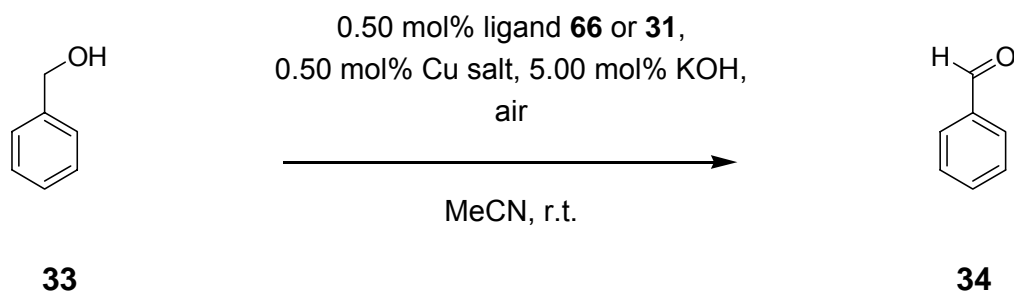
In a 25 mL round-bottomed flask, 49.0 µmol (0.50 mol%) of ligand **66** or **31** and 49.0 µmol (0.50 mol%) of copper(I) chloride or copper(II) acetate were added to 10.0 mL of acetonitrile. To this reaction mixture, 100 µL of diphenylether were added as the internal standard, then 1.00 mL (9.70 mmol, 970 mM, 1.00 eq) of benzyl alcohol **33** was added, followed by 27.5 mg (490 µmol, 5.00 mol%) of potassium hydroxide. The reaction mixture was stirred at room temperature with the flask open to air for 3 hours. 100 µL of solution was taken, 1.00 mL of acetonitrile was added and the resulting solution was filtered over a short aluminium oxide (pH neutral) column. The filtrate was analysed by GC (GC program 1).

Table 8-14: **Yield and turnover after 3 hours for the catalytic aerobic oxidation of benzyl alcohol 33 with ligands 66 and 31.**

entry	ligand			copper salt			yield of benzaldehyde[a]	TON
		[mg]	[mol%]		[mg]	[mol %]	[%]	
1	**66**	34.0	0.50	Cu(II)(OAc)$_2$	8.75	0.50	18	36
2	**31**	21.1	0.50	Cu(II)(OAc)$_2$	8.75	0.50	17	34
3	**66**	34.0	0.50	Cu(I)Cl	4.90	0.50	18	36
4	**31**	21.1	0.50	Cu(I)Cl	4.90	0.50	19	38
5	**66**	34.0	0.50	-	-	-	2	4
6	-	-	-	Cu(II)(OAc)$_2$	8.75	0.50	4	8
7[b]	**66**	34.0	0.50	Cu(II)(OAc)$_2$	8.75	0.50	1	2
8[b]	**66**	34.0	0.50	-	-	-	1	2
9[b]	-	-	-	Cu(II)(OAc)$_2$	8.75	0.50	1	2
10	-	-	-	-	-	-	1	2
11[b]	-	-	-	-	-	-	1	2

[a] Benzoic acid was not formed.

[b] The reaction was carried out without potassium hydroxide.

8.11.1.2 Oxidation of 3-methoxybenzyl alcohol **39** to 3-methoxybenzaldehyde **40**

I-MAG-46
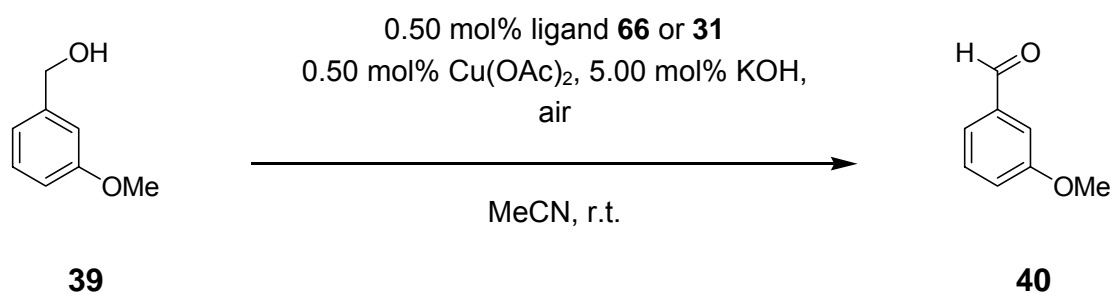
In a 25 mL round-bottomed flask, 8.00 mg (140 µmol, 5.00 mol%) of potassium hydroxyde, 2.50 mg (14.0 µmol, 0.50 mol%) of copper(II) acetate and 14.0 µmol

(0.50 mol%) of ligand **66** or **31** were suspended in 25.0 mL of acetonitrile. 350 μL (2.80 mmol, 112 mM, 1.00 eq) of 3-methoxybenzyl alcohol **39** were added to the solution and the reaction mixture was shaken at room temperature with the flask open to air for 4 hours. In order to follow the reaction, aliquots of 50.0 μL were taken, added to 5.00 mL of acetonitrile and 50.0 μL of the resulting solution were transferred to a 384-well titerplate for analysis at λ = 310 nm.

Table 8-15: **Yield and turnover after 3.5 hours for the catalytic aerobic oxidation of 3-methoxybenzyl alcohol 39 with ligands 66 and 31.**

entry	ligand	[mg]	[mol%]	3-methoxybenzaldehyde[a) [%]	TON
1	**66**	9.70	0.50	16	32
2	**31**	6.00	0.50	13	26

[a) 3-Methoxybenzoic acid was not formed.

Table 8-16: **Formation of 3-methoxybenzaldehyde 40 by catalytic aerobic oxidation with ligands 66 and 31.**

time [mins]	yield [%]	
	ligand **66**	ligand **31**
10	6.9	3.3
20	8.0	7.4
40	9.9	9.6
70	12.2	11.2
120	14.7	12.6
165	14.7	14.1
210	16.2	14.2
260	16.3	14.2

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10 Appendix

10.1 List of abbreviations

° C	degree celcius
μL	microliter
μmol	micromol
AA	amino acid
abs.	absolute
Ac	acetyl
Ac_2O	acetic anhydride
bipy	bipyridine
Boc	*tert*-butyloxycarbonyl
BOP	(benzotriazol-1-yloxy)-tris(dimethyl-amino)-phosphonium
BuLi	butyllithium
conc.	concentrated
CDI	1,1'-carbonyl-diimidazole
Cys	cysteine
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DIC	*N*,*N'*-diisopropycarbodiimide
DCM	dichloromethane
DEAD	diethylazodicarboxylate
DIAD	diisopropylazodicarboxylate
DMAP	4-*N*,*N'*-dimethylaminopyridine
DMF	*N*,*N'*-dimethylformamide
DMSO	dimethyl sulfoxide
eq	equivalent

Fmoc	9-fluorenylmethyloxycarbonyl
Fmoc-OSu	*o*-(9-fluorenylmethyloxycarbonyl)-*N*-hydroxysuccinimide
GC	gas chromatography
Gly	glycine
GO, GOase	galactose oxidase
h	hour
HBTU	2-(1*H*-benzotriazol-1-yl)-1',1',3',3'-tetramethyluronium hexafluorophosphate
His	histidine
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
LC–MS	liquid chromatography – mass spectrometry
min.	minute
mod-Cys	modified cysteine
MOM	methoxymethyl
NHE	normal hydrogen electrode
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
PEG	polyethyleneglycol
PG	protecting group
Phe	phenylalanine
Phen	phenanthroline
Pro	proline
py	pyridine
PyBOP	1-benzotriatolyloxy-tripyrrolidinophosphonium hexafluorophosphate
R	residue

r.t.	room temperature
t-Bu	*tert*-butyl
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TEMPO	2,2,6,6-tetramethylpiperidin-1-yloxy
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TOAC	2,2,6,6-tetramethylpiperidin-1-yloxy-4-amino-4-carboxylic acid
TON	turnover number
TSTU	*O*-(*N*-succinimidyl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate
Trt	trityl
Tyr	tyrosine
UV	ultra-violet

10.2   Abstract

Within this thesis, copper complexes have been synthesised as models for the enzyme galactose oxidase. A combinatorial library of 81 decapeptides containing the redox active TOAC amino acid was synthesised following the split-mix strategy. The peptide-ligands were complexed with copper(II) and screened for activity in the aerobic oxidation of 3-methoxybenzyl alcohol. The screening revealed active members. Moreover an unnatural amino acid derived from *L*-cysteine has been synthesised. This amino acid is a model for the Tyr-Cys cross-link found at the active site of galactose oxidase and was further included successfully in two different peptide sequences. In a third part, a low molecular weight tosylamide ligand was synthesised on solid support. The copper complex of this ligand showed activity in the aerobic oxidation of benzyl alcohols.

10.3   Kurzzusammenfassung

Im Rahmen dieser Arbeit wurden Kupferkomplexe als Modelle für Galactose-Oxidase entwickelt. Eine kombinatorische Bibliothek von 81 Decapeptiden, die die redoxaktive Aminosäure TOAC enthält, wurde mit Hilfe der Split-Mix Methode synthetisiert. Die Peptidliganden wurden mit Kupfer(II) komplexiert und auf Aktivität in der aeroben Oxidation von 3-Methoxybenzylalkohol geprüft. Im Screening konnten aktive Katalysatoren identifiziert werden. Darüberhinaus wurde eine unnatürliche, vom *L*-Cystein abgeleitete Aminosäure dargestellt. Diese Aminosäure ist ein Modell für den Tyr-Cys Cross-link der Galactose-Oxidase und wurde erfolgreich in zwei verschiedene Peptidsequenzen eingebaut. Im dritten Teil dieser Arbeit wurde ein niedermolekularer Tosylamid-Ligand auf der Festphase synthetisiert. Der Kupferkomplex dieses Liganden zeigte ebenfalls katalytische Aktivität in der aeroben Oxidation von Benzylalkoholen.

Erklärung

"Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzen Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmung dieser Promotionsordnung ist mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. A. Berkessel betreut worden."

Köln, 13.05.05

(Magali Dousset)

CURRICULUM VITAE

PERSONAL DATA

Magali Dousset

Born on 09.03.1978

In L'Isle-Adam, Val d'oise, France

French nationality

EDUCATION

09/01 – 05/05   PhD with Professor Berkessel, Institute for Organic Chemistry, University of Cologne, Germany

04/01 – 07/01   Research work in the group of Professor Jaouen, Organometallic Chemistry Department, UMR CNRS n°7576, Paris, France
Title: "Use of [1,2] Wittig transposition for the synthesis of new steroids"

06/01   Obtainment of the Maîtrise de Chimie

09/99 – 06/01   Student in Licence and Maîtrise at the University of Cergy-Pontoise, France

09/98 – 06/99   Student at Coventry University, England
Preparation of the Bachelor of Applied Chemistry (second year)

06/98   Obtainment of the DUT de Chimie

09/96 – 06/98   Student at the IUT de Chimie in Orsay, France

06/96   Obtainment of the Baccalauréat
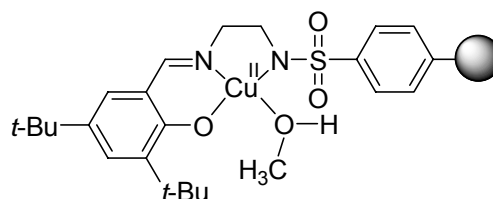
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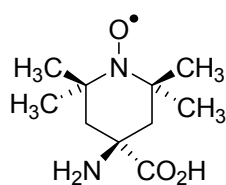
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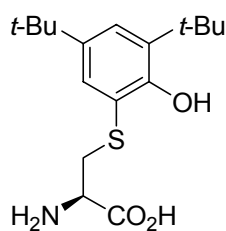
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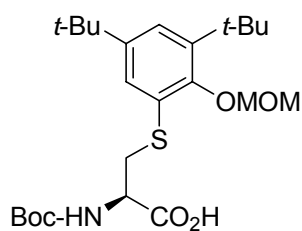
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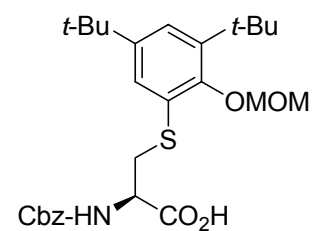
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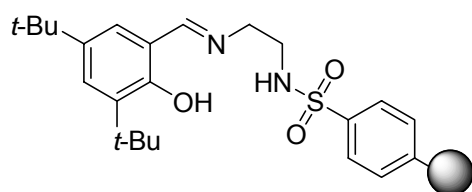
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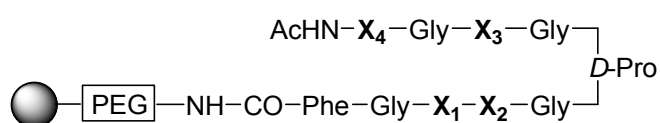
X_1 = mod-Cys	X_1 = mod-Cys
X_2 = Tyr	X_2 = His
X_3 = His	X_3 = His
X_4 = His	X_4 = Tyr
82	**83**
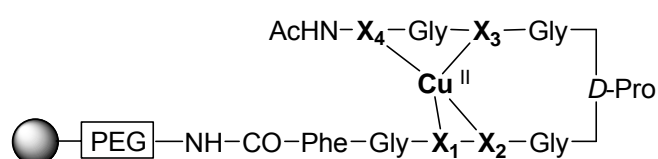
X_1 = mod-Cys	X_1 = mod-Cys
X_2 = Tyr	X_2 = His
X_3 = His	X_3 = His
X_4 = His	X_4 = Tyr
84	**85**
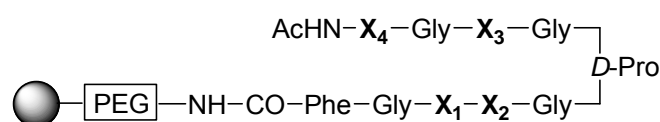
X_n = Tyr, His, TOAC

99

X_n = Tyr, His, TOAC

100