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*Synthesis of 3-hydroxypyridin-4-one-derivatives and a tripodal
branching unit as part of a dendron for the application in
photodynamic therapy*

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ii. Abstract

Photodynamic therapy (PDT) is a relatively recent medical technique to treat various types of surface tumours. It unites two non-toxic components, a photosensitizing agent and laser light of a specific wavelength to induce tissue damage through the formation of reactive oxygen species. Exogenous administration of 5-aminolaevulinic acid (5-ALA) leads to an accumulation of the photosensitive protoporphyrin IX (PpIX) within cells. This molecule is formed throughout the haem biosynthesis pathway and by increasing its intracellular amount it becomes possible to induce targeted necrosis of tumour tissue. In the last step of the haem biosynthesis pathway the enzyme ferrochelatase converts its substrate PpIX into haem by the insertion of ferrous iron. The enzyme can be effectively inhibited by bidentate iron chelators deriving from 3-hydroxypyridin-4-one (HPO). The enhanced photodynamic effect of the combined treatment with both 5-ALA and a HPO derivative has been proven in recent studies.

The synergistically acting drugs can be combined in a dendrimer to improve both the pharmacokinetics and the compliance of treatment. Dendrimers possess an increased ability to target malignant tissue and are therefore favoured molecules for the use in cancer treatment.

At the present, chemists at the department of Medicinal Chemistry at King's College London are involved in the synthesis of HPO-derivative containing dendrons for the application in PDT. The aim of this 4 months lasting Diploma thesis project was to synthesize a first generation dendron containing three ester bonded terminal HPO-derivative moieties. A carbon chain length of six or eight between the pyridinone ring and the ester grouping of the branching unit had been previously proven to be most appropriate in terms of lipophilicity and cellular uptake.

The two benzyl-protected iron chelators were prepared from ethyl maltol in two steps applying efficient and high-yielding procedures. The first attempts to establish a triol building block, consisting of a protected β -alanine linking unit and a tripodal branching unit, could not be successfully achieved. Using the tripodal branching unit without the protection of its hydroxyl groups, difficulties occurred when it was tried to couple it to β -alanine via amide bond formation. Having investigated different types of protective groups for both the triol branching unit and β -alanine, a four-step synthetic route finally led to the triol building block

in a good yield. The introduction of acetate esters as hydroxyl protective groups allowed the amide coupling and afforded the desired compound upon saponification of the acetates. The last step consisted of coupling the HPO-derivatives to the erected building block through ester bond formation and could only be partially achieved due to the limited time that was available for the project.

In following synthetic attempts it will be tried to modify the conditions of this last reaction and to synthesize further similar dendrons that can be assembled to dendrimers together with 5-ALA containing compounds for tests in biological studies.

iii. Zusammenfassung

Unter der photodynamischen Therapie (PDT) versteht man ein relativ neuartiges medizinisches Verfahren zur Behandlung von oberflächlich liegenden Tumoren. Das photodynamische Wirkprinzip vereint - mit einem photosensitiv wirksamen Agens und Laserlicht spezifischer Wellenlänge - zwei nicht-toxische Elemente in einer Art und Weise, dass durch die Bildung reaktiver Sauerstoffspezies (ROS) gezielt Gewebszerstörung induziert werden kann. Das exogene Zuführen von 5-Aminolävulinsäure (5-ALA) führt zum Anstieg des intrazellulären Protoporphyrin IX (PpIX) Spiegels. Dieses photosensitive Zwischenprodukt der Haem Biosynthese ermöglicht bei Bestrahlung mit Licht geeigneter Wellenlänge die zielgerichtete Zerstörung von Tumorgewebe. Im letzten Schritt des Biosynthesewegs verwandelt das Enzym Ferrochelatase sein Substrat PpIX durch den Einbau eines Fe^{++} Atoms in Häm. Zweizählige Eisenchelatoren vom Typ der 3-Hydroxy-Pyridin-4-one (HPO) sind in der Lage, dieses Schlüsselenzym effektiv zu inhibieren. In aktuellen Studien wurde gezeigt, dass die Kombinationstherapie mit 5-ALA und einem HPO Derivat den photodynamischen Effekt um ein Vielfaches verstärken kann.

Vereint man die beiden synergistisch wirksamen Pharmaka in einem einzigen Molekül, einem Dendrimer, dann verbessert dies sowohl die Pharmakokinetik der Wirkstoffe als auch die Compliance der Therapie. Dendrimere verfügen durch ihre außergewöhnliche Struktur die Gabe, spezifisch in Tumorzellen zu akkumulieren und eignen sich dadurch exzellent für die Anwendung in der Krebstherapie.

Derzeit befasst sich eine Arbeitsgruppe des Departments für Medizinische Chemie am King's College London mit der Synthese von Dendronen für die Anwendung in der PDT. Diese tragen HPO-Abkömmlinge als Endgruppen und werden im Zuge einer Zusammenarbeit mit der University of Essex gemeinsam mit 5-ALA enthaltenden Dendronen zu Dendrimeren zusammengesetzt.

Ziel dieses Projekts im Rahmen der Diplomarbeit war die Synthese eines Dendrons erster Generation, welches drei terminale HPO-Derivative trägt, die über von den Pyridinringen ausgehenden Alkyl-Spacern durch Esterbindungen an eine dreifüßige Verzweigungseinheit gekoppelt sind. Ausgehend von Ethylmaltol wurden nach Schützung der Hydroxygruppe als Benzylether die Umwandlung in Pyridinderivate durch Umsetzung mit 6-Aminohexansäure und 8-Aminooktansäure in guten Ausbeuten erreicht.

Die ersten Versuche, einen geschützten β -Alanin-Linker über eine Amidbindung an eine ungeschützte Triol-Verzweigungseinheit zu knüpfen, waren nicht erfolgreich. Es wurden unterschiedliche Schutzgruppen sowohl für das Triol als auch für β -Alanin getestet und nach einigen Versuchen führte ein 4-stufiger Syntheseweg zum gewünschten Dendron-Block mit drei terminalen Hydroxygruppen. Als Schutzgruppen für die Triol Verzweigungseinheit wurden Acetatester eingeführt, welche nach erfolgreicher Amidkopplung an den β -Alanin-Linker einfach durch Verseifung entfernt werden konnten. Der gewünschte Dendron Block wurde in guter Ausbeute erhalten. Der letzte Schritt, in dem diese Triol Einheit durch Esterbindungen an die HPO-Endgruppen geknüpft hätte werden sollen, war nur teilweise erfolgreich.

Aufgrund des limitierten Zeitraums, der für den Erasmus-Aufenthalt zur Verfügung gestanden ist, finden derzeit am King's College London noch weitere Modifikationen an diesem letzten Syntheseschritt statt, um zu das gewünschten Dendron in ausreichender Menge und Reinheit zu erhalten. Weitere ähnliche Dendrons sollen schlussendlich mit 5-ALA beinhaltenden Abschnitten zu Dendrimeren zusammengefügt und auf ihre biologische Aktivität überprüft werden.

1 Photodynamic therapy

The beneficial effects of phototherapy have been known by humans for more than three thousand years, but it was only in the past century that light in combination with photosensitizing agents has been used in oncology to treat various kinds of cancer. The term “Photodynamic Therapy” was first introduced in 1904 by the German scientist Hermann von Tappeiner, who discovered photodynamic action by chance when one of his students tested the toxic side effects of the dye acridine on paramecia under different light conditions. They found, that there exists a connection between the activation of the dye with light and the survival of the cells. From then onwards numerous scientists attempted to treat malignant tumors and diseases of the skin with the help of photosensitizing agents and light in the wavelength range absorbed by the photosensitizer^[1].

1.1 Mechanism of action

Photodynamic therapy (PDT) is a cancer treatment that unites two non-toxic components, a photosensitizing agent and laser light of a specific wavelength, to induce tissue damage in a very precise way through the formation of reactive oxygen species (ROS). The photosensitive drug, usually a tetrapyrrolic macrocycle, is administered either intravenously or topical to the patient. After having equilibrated in the body, the photosensitizer accumulates in tumor cells with a remarkable selectivity towards normal cells. It is not completely understood why tumor cells and rapidly proliferating cells retain photosensitizers longer and in a higher amount compared to healthy tissue. This selectivity is probably due to the fact that the malignant tissue is surrounded by more blood vessels, less lymphatic drainages and furthermore shows a lower pH value of the interstitial fluid^[2].

Because the ratio of the accumulated photosensitive drug and therefore also the fluorescence is higher in malignant cells than in healthy tissue, a tumor can easily be detected by irradiation with red laser light^[3]. There are two photophysical mechanisms in PDT that are responsible for the anti-cancer effects and both of them involve oxygen.

The type II mechanism (Figure 1) is defined as an *energy transfer process* and it is believed to be the main reason for the oxygen-dependent phototoxicity of the drugs that are used in PDT. Once the tissue is radiated with red laser light, the photosensitizer absorbs a certain

amount of energy and is then promoted from its ground state into an extremely unstable singlet excited state. The activated drug molecule then experiences *intersystem crossing* to form a longer lived triplet excited state. From this state, the photosensitizer reacts with intercellular molecular oxygen and decays back to its ground state. At the same time singlet oxygen is created, which is the actual photodynamic agent. The singlet oxygen attacks lipids or proteins to form other reactive intermediates and radicals, which can further react with cellular biomolecules ^[1, 3-4].

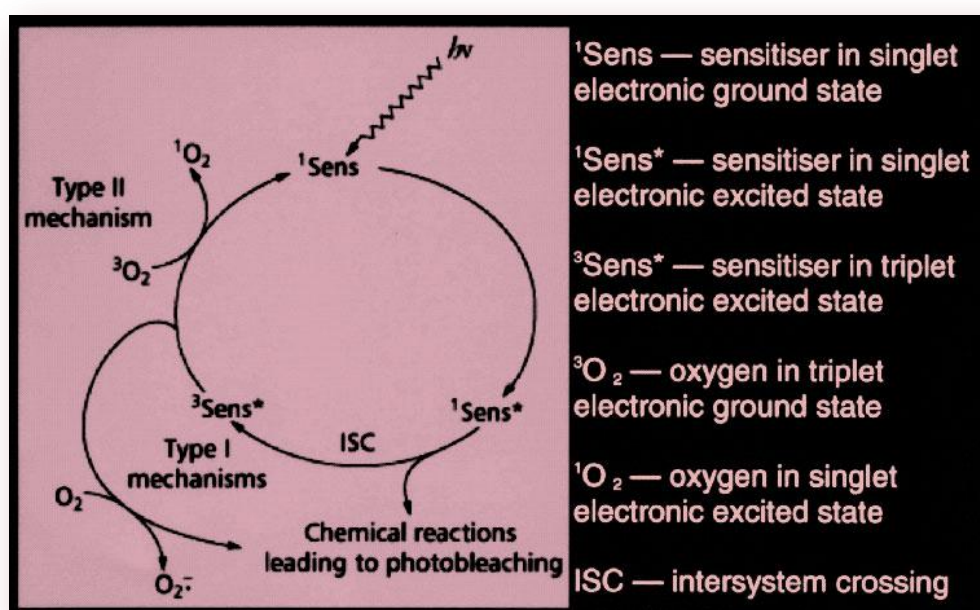


Figure 1: Photophysical mechanisms involved in PDT^[3]

The type I mechanism (Figure 1) is defined as a *radical* or *redox reaction* which involves a transfer of electrons or hydrogen atoms between the photosensitizer, excited to its triplet state, and neighboring molecules. This transfer results in radical intermediates which are able to initiate further chain reactions that lead to the phototoxic degradation of other biomolecules and cell death. In contrary to the type II pathway, the type I mechanism can also take place without the presence of oxygen ^[1, 3-4].

1.2 The effect of photosensitizers on tumors

The mechanisms of PDT-mediated cellular damage are complex and the knowledge about these processes is still limited. There exist three main mechanisms that induce tumor-tissue destruction. They are interdependent and combine a number of biochemical and molecular factors.

Direct tumor-cell killing, one of these mechanisms, correlates to the homogenous distribution of the photosensitizer within the tumor as well as to the amount of oxygen that is present in the tissue. The decrease in the available amount of oxygen is a result of its constant consumption during the photochemical process and of the immediate response to the illumination with light.

Vascular damage of the surrounding blood vessels is another reason for the efficacy of PDT. The tumor relies on their provision of nutrients and it has been reported that PDT leads to a microvascular collapse which results in both hypoxia and anoxia.

According to Mladen Korbely and his colleagues, the *immune host response* is the reason for the complete elimination of cells that survive the effect of the photodynamic treatment in the first place. The production of inflammatory cytokines is upregulated as a response to PDT, furthermore it has been reported that lymphocytes, leukocytes and macrophages infiltrate the tissue in a higher amount after such treatment^[5].

1.3 Light irradiation and sources of light used in PDT

The choice of a suitable device as the source of light depends on the light absorption maximum of the photosensitizing drug. One of the first drugs used in clinical PDT was haematoporphyrin derivative (HpD), which is activated when illuminated with light in the near-UV, at a wavelength of around 400 nm. Unfortunately, the living tissue which is penetrated by laser-light, effectively absorbs at this exact wavelength and therefore reduces the effectiveness of the treatment. An ideal photosensitizing drug is thus activated at wavelengths > 600 nm in order to ensure a better absorption of tissue-penetrating red-light. Light at these wavelengths also holds the advantage of being able to reach deeper-lying tumors^[3]. Due to this circumstance light sources with an output of light at the red region of the spectrum and an improved tissue penetration have been introduced to PDT.

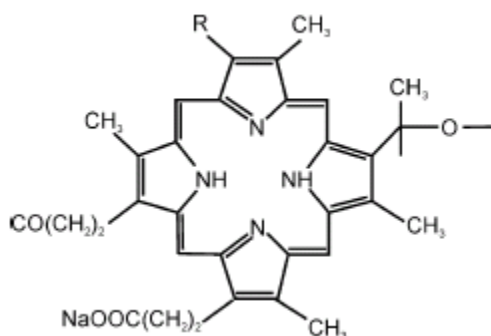
Initially, regular gas discharge lamps were used in PDT. They could only be applied to easily accessible parts of the skin and to superficial lesions. It was the revolutionary introduction of lasers attached to optical fibers that enabled an endoscopic light-delivery to reach nearly every organ of the human body. Today a wide range of lasers and lamps are available. The choice of the most suitable light source depends on many factors, such as the targeted organ, the type of photosensitizer, the ease of use and cost. The advantage of lasers as a source of light is that they are powerful and deliver monochromatic light at a distinct wavelength. The selection of the appropriate wavelength is crucial and has to correlate to the maximum absorption band of the photosensitizer. Due to these prerequisites, one laser can only be used in combination with a certain photosensitizing drug. Lamps, on the other hand, provide a wider spectrum of wavelengths and are often combined with a variety of filters to select specific wavelengths. They are easier and cheaper in use, but can only be applied to lesions on the skin^[6].

1.4 Photosensitizers in clinical PDT

Although many chemical compounds can act as photosensitizers, only a few possess the correct properties to render them suitable for clinical trials. In clinical practice, there are three main groups – porphyrins, chlorophylls and dyes – from which photosensitizers derive. All clinically useful compounds are able to target malignant tissue and to be activated by light at a high enough wavelength that is appropriate for therapy.

1.4.1 The porphyrin family^[7]

Photophrin® (Haematoporphyrin derivative (HpD), Formula 1) is the first systemically studied and most commonly used photosensitizer in PDT. Haematoporphyrin is obtained from acid-hydrolyzed haem or haemoglobin and is then treated with further chemicals to result in a mixture of monomers, dimers and oligomers of the original compound. The complex combination of these derivatives is crucial for their efficacy.

Formula 1: Molecular structure of Photophrin[®][7]

Photophrin[®] is approved all over the world to treat different kinds of cancer, such as esophageal tumors, early-stage lung cancer and bladder cancer. This drug is relatively non-toxic, reliable and most importantly, the treatment is pain-free. On the other hand, there are quite a few reasons, why HpD is not the ideal photosensitizer: the drug accumulates also in healthy tissue to a comparatively high level and is only slowly cleared from the system. This leads to a cutaneous photosensitivity to solar radiation, which sometimes lasts up to 4 weeks or longer.

Another member of the porphyrin family is Verteporfin, a benzoporphyrin derivative that is used in a formula together with liposomes. It is activated at a higher wavelength than HpD and is therefore able to penetrate more deeply into the skin. Due to the fact that it accumulates in the targeted tissue very fast and is cleared rapidly, the risks of post-treatment photosensitization of the skin are comparatively low.

A further subclass of the porphyrin-family is texaphyrin. This photosensitizer, which contains the paramagnetic metal gadolinium(III), selectively targets tumor-tissue and is detectable by magnetic resonance imaging (MRI). It has been used to support MRI scanning for a few years and as it is activated by light at a comparatively high wavelength, it is also a promising compound for clinical PDT^[8].

5-Aminolevulinic acid (5-ALA) is an endogenously occurring amino acid that represents the first step in the porphyrin synthesis pathway. Porphyrin metabolism leads to the production of haem in mammals. The photosensitive intermediate protoporphyrin IX is formed at a higher rate when 5-ALA is administered topically or systemically. The important role of 5-ALA and its derivatives in PDT will be discussed in more detail in section 1.5..

1.4.2 The chlorine family^[7]

One of the members of the heterogeneous group of chlorine derivatives is Foscan[®] (Temoporfin). It possesses a high efficiency in light-converting and the duration of treatment declines to only a few minutes. The compound is used in chemotherapy for the treatment of various cutaneous lesions and tumors in the head and in the neck. However, there are clinical drawbacks: the systemic injection of the drug is associated with pain and due to its high activity, patients are photosensitive after treatment even to normal room-light.

Purpurins, also belonging to this group, are chemically modified chlorophyll-derivatives and are currently used for the treatment of Kaposi sarcoma, basal cell and chest wall metastasis. Mono-L-aspartyl-chlorin e6 is another multifunctional and safe photosensitizer, but it lacks of tissue selectivity, especially at the dosage needed in PDT. The chlorine-based drug photochlor (HPPH), being effective and safe, shows high potential to become a clinical photosensitizer.

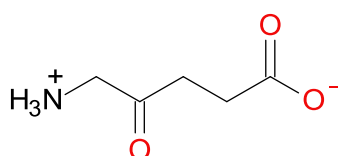
1.4.3 The dye family^[7]

Out of the commonly used dyes, phthalocyanines and their relatives have the highest ability for clinical PDT. They are activated at wavelengths between 650 and 850 nm and because of their lipophilic character they are dependent on liposomal formulation. All of the clinically successful compounds are chemically well defined and similar to porphyrin. Their efficacy can be enhanced when they are connected to metals to form complexes.

Photosens, a mixture of sulfonated aluminium phthalocyanines, has been used in clinical trials to treat various kinds of cutaneous and endobronchial lesions.

1.5 5-Aminolevulinic acid in photodynamic therapy^[9]

In mammals, porphyrins are synthesized mostly in erythropoietic tissue and the liver. Throughout the haem biosynthetic pathway (Figure 2) they lead to the production of haem.



Formula 2: 5-Aminolevulinic acid (5-ALA)

5-Aminolevulinic acid (5-ALA, Formula 2) plays an important role in this pathway, as it is the endogenous precursor for the formation of porphyrins. In the first step, glycine and succinyl coenzyme A (CoA) are condensed to form 5-ALA. This reaction is catalyzed by the enzyme ALA synthase, which is located in the inner membrane of the mitochondria. The cytoplasmic enzyme ALA dehydratase then combines two molecules of 5-ALA to create porphobilinogen (PBG), the first intermediate of the pathway that includes a pyrrole ring. After that, four molecules of PBG are condensed to form a linear tetrapyrrole intermediate, which is subsequently brought to yield in the cyclic uroporphyrinogen III. The enzymes responsible for these steps are PGB desaminase (PGBD) and uroporphyrinogen III cosynthase (Figure 2). Four acetic acid carboxylic groups are removed from uroporphyrinogen III by the cytoplasmic uroporphyrinogen decarboxylase. The resulting molecule, coproporphyrinogen III, is further oxidized and decarboxylated by a mitochondrial enzyme to yield protoporphyrinogen IX. Protoporphyrinogen oxidase then removes six hydrogen atoms in the tetrapyrrole ring to form protoporphyrin IX (PpIX). Only these oxidized porphyrins are intensely colored. In the last step the enzyme ferrochelatase, which is embedded in the inner membrane of the mitochondria, inserts iron into the tetrapyrrole ring of PpIX to form haem ^[9-10]. The 5-ALA derived PpIX is able to act as a photosensitizer when it is activated by light at a suitable wavelength.

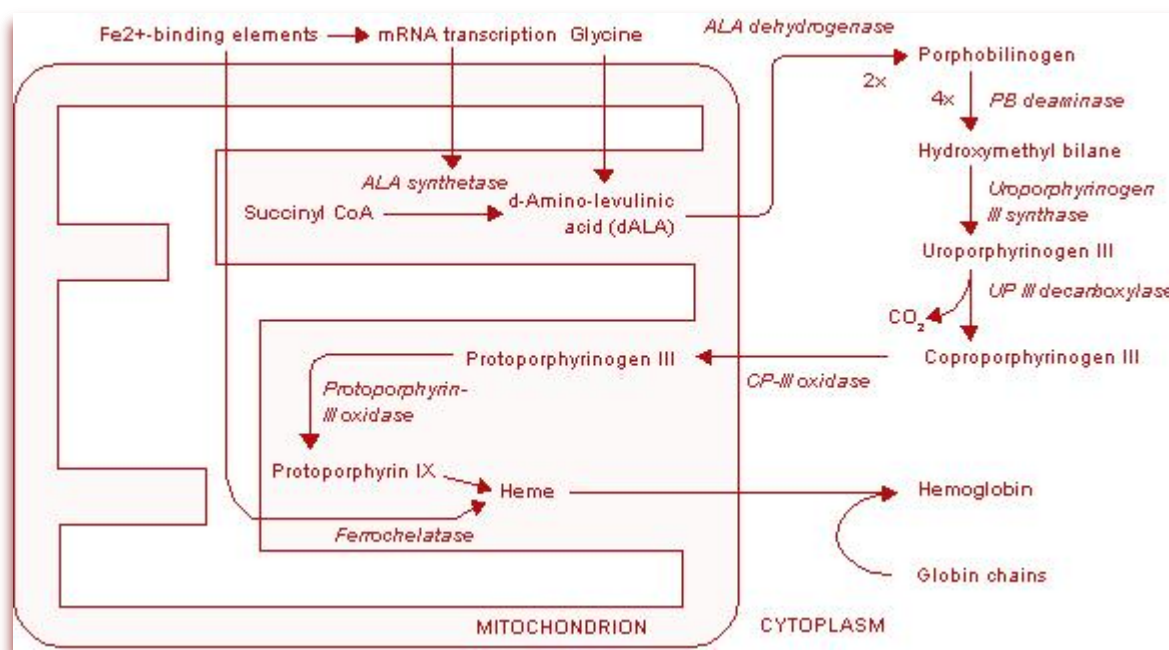


Figure 2: The haem biosynthetic pathway^[11].

The regulation of haem synthesis

Haem biosynthesis is regulated by the availability of the substrates as well as by the inhibition of the first enzyme in this pathway, the ALA synthase. These controlling mechanisms vary between the liver and the bone marrow. Depending on the type of tissue, there exists either an erythroid-specific or a housekeeping isoenzyme for the ALA synthase, which are encoded by different genes. These enzymes, which show a quite low activity, can be directly inhibited through negative feedback regulation by haem. The intracellular concentration of haem can also influence the rate of transcription, translation and the transportation of the enzyme to the mitochondria.

Topical application of 5-ALA

After about 4 to 14 hours of the topical application of ALA, the formed PpIX can be activated with light at a wavelength of approximately 400 nm and then be measured with spectrofluorometric methods. Depending on the concentration of ALA in the formula and on the duration of the exposure, PpIX is either only produced on the treated sites of the skin or in larger amounts it can also lead to a generalized photosensitivity. As an adverse reaction, most of the patients suffer from an itching and burning feeling at the light-exposed areas of the skin which can last up to a few hours after the treatment. This side effect can be diminished when lignocaine in various concentrations is used either formulated in a cream or as an intracutaneous anaesthetic.

Systemic administration of 5-ALA

It is not completely clear whether the exogenous administration of 5-ALA is toxic. It has been reported, that the systemically applied drug can cause a temporary feeling of nausea and sometimes leads to transitory abnormal liver functions. Neurotoxic effects have not been observed below a dose of 60 mg/kg for oral and 30 mg/kg for intravenous administration. Despite the fact, that the 5-ALA plasma levels of cancer patients in PDT treatment are much higher than the plasma levels of porphyric patients, they do not suffer from the typical symptoms that are related to porphyria (severe neurologic deficits, vomiting, seizures). The reason for this phenomenon is not clear and careful use of the drug is recommended when people with porphyria and liver or kidney problems are treated systemically with 5-ALA.

While the treatment of large lesions and tumors with systemically administered 5-ALA needs to be improved, ALA-based PDT appears to be an excellent choice for the treatment of smaller and more superficial malignant lesions of the aerodigestive tract, for instance dysplasia in the esophagus.

1.6 Iron chelators in photodynamic therapy

The enzyme ferrochelatase catalyses the last step of the haem biosynthesis (Figure 2 + Figure 3). Haem is formed by the insertion of ferrous iron (Fe^{++}) into the tetrapyrrole macrocycle of protoporphyrin IX (PpIX). The mechanism by which ferrochelatase catalyses this incorporation is only poorly understood. Ferrochelatase is located in the inner membrane of the mitochondrion and it is suggested that the porphyrins bind to an identified cleft, which also includes the metal-binding site of the enzyme^[12].

PpIX acts as a photosensitizer in photodynamic therapy and can be produced at a higher rate if its precursor 5-ALA is exogenously administered. Inhibiting the enzyme ferrochelatase by limiting the intracellular iron supply is another way to increase the amount of PpIX. If the substrate is not readily available, the formation of haem is hindered and PpIX accumulates within the cells. Both of these approaches, the excessive administration of 5-ALA and the inhibition of ferrochelatase, enhance the formation of PpIX and therefore increase the effectiveness of photodynamic therapy^[13].

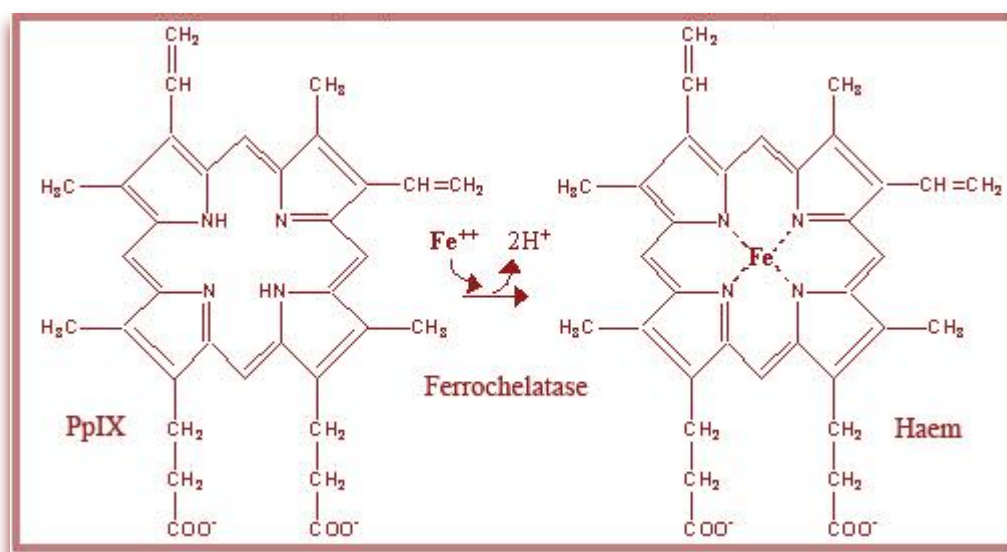


Figure 3: Insertion of iron via ferrochelatase^[14]

1.6.1 The enzyme ferrochelatase

Ferrochelatase is a key enzyme in the human haem biosynthesis pathway and its function closely correlates to the available amount of ferrous iron within the mitochondria. The protein consists of two identical domains and each of the subunits contains an iron-sulphur cluster. The enzyme's association with the inner membrane of the mitochondria is achieved by the formation of a hydrophobic lip which also represents the entry to the active binding site of the enzyme^[15]. It is proposed that the active site binds the substrate PpIX tightly and buries it completely to form an encapsulated hydrophobic pocket^[16]. Hansson et al. investigated the properties of the ferrochelatase of *Bacillus subtilis* in order to gain better knowledge about the metal-binding residues for ferrous iron in the active site. They used site-directed-mutagenesis to prove that two conserved amino acids within the binding site, a histidine and a glutamic acid, strongly influence the enzyme's affinity for iron. They found that the ferrous iron in the active site is coordinated by both of these amino acid residues and by three water molecules^[17].

Ferrochelatase and also the conversion of PpIX into haem can be inhibited by chelating the ferrous iron in the active binding site of the enzyme. Once the metal is chelated forming a stable ternary complex, the substrate's access to the active cleft is denied and PpIX accumulates within the mitochondria. Considering the fact that the enzyme is partially embedded in the membrane and therefore surrounded by a lipophilic environment, only iron-chelating agents possessing a comparatively hydrophobic character are able to reach the described metal-binding site.

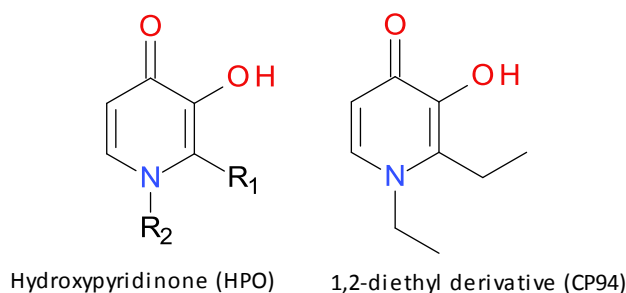
1.6.2 Regulation of iron absorption and transport

Iron is an essential metal for all eukaryotic cells and its uptake is regulated by various transport systems throughout the body. It can be obtained from diet by absorption in the gastrointestinal tract as well as from recycling old or damaged erythrocytes. In the blood serum, iron is bound to transferrin and internalized by cells via receptor mediated endocytosis. Within the cells, the metal is stored as a complex with the protein ferritin. The larger quantity of iron in the body is located in the hemoglobin molecules of erythrocytes and it is therefore necessary to make the intracellular iron available to the enzyme ferrochelatase in the mitochondria. A sufficient iron supply in the mitochondria is not only required for generating haem, but also for the biosynthesis of various iron-sulfur cluster-

containing proteins. The exact mechanism of iron uptake into the organelles is not known, but it is suggested that it is driven energetically by a membrane potential along the inner mitochondrial membrane without the further need of ATP. The reduced (ferrous) form of iron is received by the ferrochelatase directly from the inner membrane and then inserted into PpIX^[18].

1.6.3 Inhibition of mitochondrial ferrochelatase

Iron chelating compounds have mainly been used for the treatment of diseases that lead to iron overload within the human body. As an example, people who are suffering from β -thalassemia and are treated with regular blood transfusions, accumulate iron in their system. As there are no mechanisms for iron excretion in humans, it is crucial for these people to be concomitantly treated with iron chelators. Desferrioxamine, a drug deriving from a bacterial siderophore, has been widely used in such cases. Because of its high molecular weight and hydrophilic behavior it has to be administered by injection and therefore lacks good compliance. To overcome these disadvantages, orally active bidentate iron chelators, 3-hydroxypyridin-4-ones (HPOs, Formula 3) have been developed^[19].



Formula 3: Chemical structure of HPO and CP94

Smith and coworkers conducted a study in which they added the orally active iron chelator 1,2-diethyl-3-hydroxypyridin-4-one (CP94, Formula 3) to the drinking water of genetically modified mice. They observed the accumulation of porphyrins in the animals' livers after one week of treatment. CP94, which belongs to the family of HPOs, has a low molecular weight and due to its lipophilicity it is able to penetrate through bio-membranes quite easily. The results of their experiments indicate that CP94 specifically chelates iron pools available to the ferrochelatase which leads to an increased intracellular amount of protoporphyrin. They assume that this chelator suppresses the mitochondrial iron deposit and also the transport

of the metal into the mitochondria. Besides the depletion of the ferrochelatase's substrate, there is another explanation for the inhibition of the enzyme: the iron from the iron-sulphur cluster in the C-terminal area of the protein could be removed by the chelator and result in an inactive enzyme^[20].

In the same year, Si-Chung Chang and his group investigated the influence of CP94 on the amount of intracellular PpIX in different layers of the bladder after having intravesically administered 5-ALA to rats^[21]. They found an enhanced formation of PpIX when CP94 was dosed to the animal and suggested various explanations for this phenomenon. In contrast to the hydrophilic desferrioxamine, members of the HPO family are lipophilic and therefore able to access intracellular iron pools more rapidly. Because iron is essential for the cell's homeostasis, an interruption of the metal-metabolism can have antiproliferative and cytotoxic effects on malignant cell lines (for instance the metalloenzyme ribonucleotide reductase, which is a key enzyme in DNA synthesis, is also dependent on the availability of iron)^[21].

In photodynamic therapy, the dose of orally tolerated 5-ALA is limited to 60 mg/kg. According to Curnow et al., the induced PpIX fluorescence within the mucosa of rat colons can be doubled if CP94 is administered together with 5-ALA. The subsequently produced area of necrosis was found to be three times larger than without the addition of chelator^[22].

These observations were confirmed more recently by Pye et al. in 2008. They performed in vitro measurements of methyl-aminolevulinic acid induced-PpIX enhanced by CP94 on human cultured cells of dermatological origin and also in vivo studies including patients with nodular basal cell carcinoma. It was found that CP94 effectively increased the PpIX fluorescence in both circumstances. With this approach it becomes possible to reach also deeper layers of lesions. Thus, administering the same amount of 5-aminolevulinic acid, the additional CP94 is able to improve the photodynamic effect in thicker tumors. The antiproliferative effects of iron chelators, that have been previously shown, have not been proved in this study. This is probably due to the fact that CP94 is excreted rapidly by glucuronidation and all the effects on DNA synthesis are only of short duration^[23].

All these investigations support the suggestion that the hydrophobic HPO-derivative CP94 is able to successfully inhibit ferrochelatase by chelating iron in the binding site of the molecule. As a consequence, the intracellular level of PpIX is increased. Together with the exogenous administration of the precursor 5-ALA, this leads to an enhanced effect in photodynamic therapy.

2 Dendrimers

Traditionally, polymer chemists have focused on the synthesis of linear polymers. These molecules are widely used in everyday-life (for instance in plastics, fibres, adhesives, paints etc.). However, highly branched macromolecules, adopting a spherical shape, possess very different properties. Dendritic polymers are well-defined nanostructures with unique chemical properties and therefore used in a wide range of biomedical and industrial applications. Vöglte and his coworkers introduced dendrimer chemistry in 1978 by synthesizing “cascade molecules”. Tomalia et al. first published the synthesis of “starburst-dendritic macromolecules” by using a divergent approach. “Dendrimer”, which is the currently international accepted term, originally derives from the Greek words *dendron* (tree) and *meros* (part). Synonymous terms for this type of macromolecules are “arborols” and “cascade molecules”.

A dendrimer (Figure 4) typically consists of three main structural components: a core, branches and end groups. Through successive sequences of stepwise reactions, more and more layers are added to the core molecule. Every additional layer leads to a higher generation dendrimer, which has double the number of end groups and about double the molecular weight ^{[24],[25],[26]}.

A dendron (Figure 4) is just one section of a dendrimer and usually shaped like a wedge. It consists of multiple end groups and a single reactive site at the focal point. By attaching dendrons which contain different types of terminal groups to one centered core, it becomes possible to synthesize dendrimers with extended chemical properties ^[27].

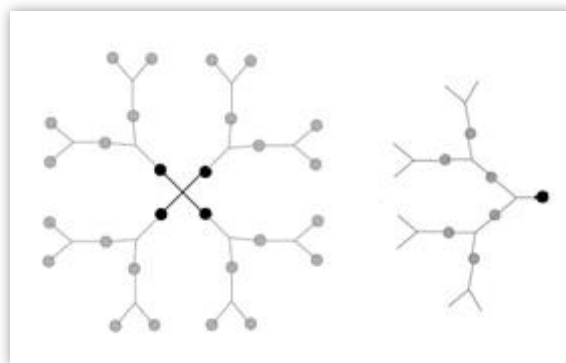


Figure 4: Left side: dendrimer, right side: dendron ^[26]

2.1 Synthesis of dendrimers

There are two main strategies for dendrimer synthesis, *divergent synthesis* and *convergent synthesis*. Ideally, the size and the number of branches can be controlled by precise synthetic processes. When core, branches and end groups are featured with different chemical characteristics, it is also possible to regulate properties like thermal stability and water solubility. However, with either of the two main methods it is difficult to synthesize pure and inexpensive molecules, because the reaction procedure requires a high number of precise reactions which are problematical to bring to completion. Only a few compounds are commercially available.

In the *divergent* dendrimer growth method (Figure 5), the dendrimer is assembled outwards from a multifunctional core. A first generation dendrimer results from the reaction of the core molecule with suitable monomers. These monomers feature one reactive site and two or more protected or dormant groups. In subsequent steps, more monomers can be attached to the activated periphery of the first generation molecule. In this way, the dendrimer can be built layer by layer to form a highly symmetrical globule. Even though the divergent method can be used to synthesize large amounts of dendrimers, there are a few drawbacks. A major problem of this approach is, that as the molecule increases in size, the growth steps can become incomplete and those excess reagents may become necessary. Under such conditions considerable quantities of side-products accumulate, which causes difficulties in the isolation procedure of the desired compound ^[25-26].

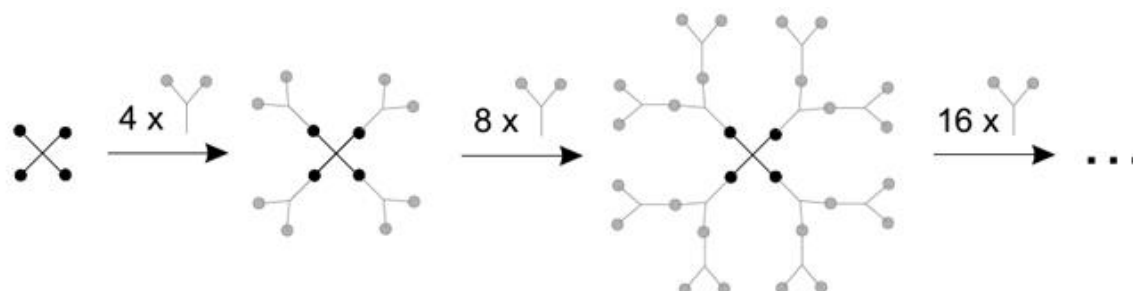


Figure 5: Schematic of divergent synthesis of dendrimers^[26]

In *convergent* dendrimer growth (Figure 6), more convenient and improved methods are applied that tend to avoid the disadvantages associated with the divergent synthesis. In this method dendrimers are built from the small end molecules on the outside of the sphere and are then linked with branching units to proceed inwards. The reaction takes place only at one

site of the growing dendrimer. Once the branching units are long enough they can be attached to a multifunctional core. With this method it is comparatively easy to purify the final product and it becomes possible to modify the peripheral functionalities of the dendrimer in a more precise way. Despite these advantages there is a limitation of dendrimer size compared to that produced by divergent method. Due to steric hindrance and problems with reactions between the dendrons and the core molecule it is not easy to synthesize higher generation dendrimers using this method ^[26, 28].

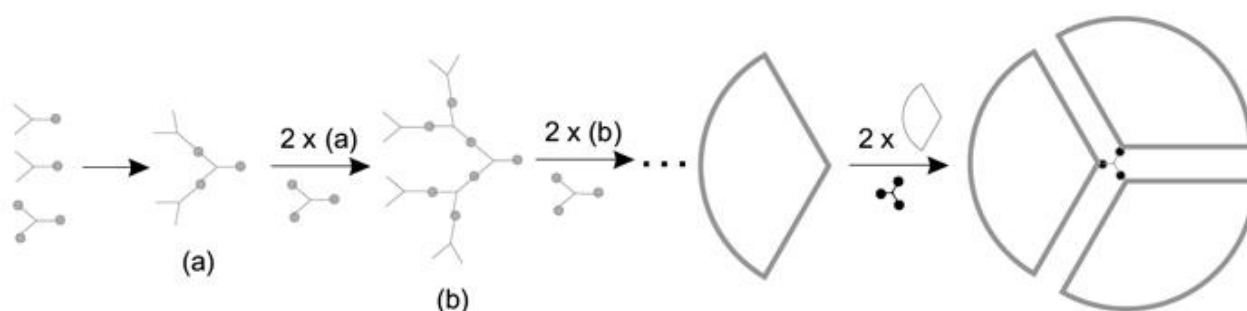


Figure 6: Schematic of convergent synthesis of dendrimers^[26]

After the development of the convergent strategy other approaches to accelerate and simplify dendrimer synthesis have been investigated. In the early 1990's Jean M. J. Fréchet and his group introduced the use of pre-assembled oligomeric species - hypercores and branched monomers. This new technique combines the advantages of the convergent and the divergent method in a way that the dendrimer can be synthesized in fewer steps and in higher yields. The double exponential and the mixed growth method (Figure 7) are further tactics that are similar to rapid growth methods of linear polymer chemistry.

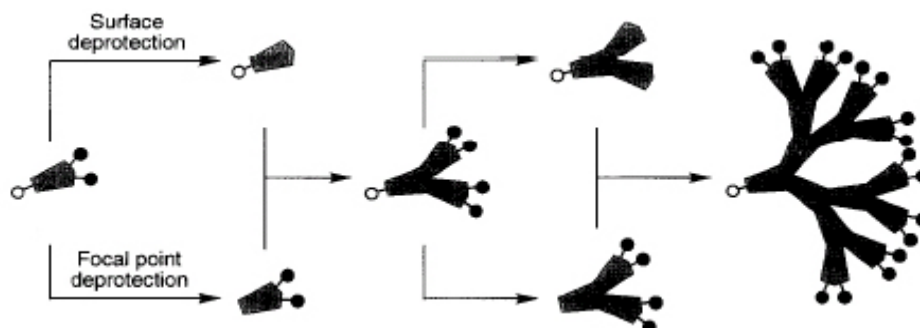


Figure 7: Double exponential growth method in dendrimer synthesis^[24]

In this approach only one branching moiety with two sites of protected functionalities is used. The separate deprotection of the surface as well as of the focal point of the branching unit leads to the formation of both a convergent-type and a divergent-type monomer. The reaction of these monomers results in an orthogonally protected trimer, which can be used for further growth steps^[29].

2.2 Dendritic conjugates of 5-ALA and HPOs for the use in photodynamic therapy

In the past decade the development of dendrimers for the use in bioscience has made an immense progress. The unique chemical properties of dendrimers render them suitable for applications in immunology and drug delivery, for instance the development of antivirals and vaccines. The synthesis of dendrimers that are biocompatible has led to new approaches especially in the field of cancer treatment, where these macromolecules seem to be the ideal drug delivery system to target tumor cells. Improved pharmacokinetics and increased photodynamic effects have been observed when low molecular weight therapeutic agents are derivatized on dendritic polymers^[30].

Dendritic conjugates of 5-ALA and iron chelators

The precursor 5-ALA that is used in PDT does not possess the ideal properties for targeting malignant cells effectively. At pH 7,4 it is zwitterionic and relatively hydrophilic which means that the molecule is not readily taken up by cells and therefore shows a comparatively poor bioavailability. This limitation led to the investigation of 5-ALA-dendrimer conjugates. Recently, Battah et al. have been able to synthesize dendrimers bearing 18 5-ALA residues that showed improved pharmacokinetics and higher cellular uptake. The level of the photosensitizing PpIX within tumorigenic cells could be enhanced and a corresponding increase in light-induced cell destruction was measured^[31].

Not only the poor bioavailability of the prodrug 5-ALA, but also the conversion of the endogenously formed photosensitizer PpIX into haem has limiting effects on the efficiency of photodynamic therapy. As described previously, the combination of 5-ALA with iron-chelating compounds can synergistically enhance PpIX levels caused by the inhibition of the

enzyme ferrochelatase. However, the main disadvantage of combining 5-ALA and HPO-derivatives is the necessity of separately administering two drugs.

In order to improve both the pharmacokinetics and also the compliance of this treatment, the properties of 5-ALA and HPO conjugates were investigated. In recent proof-of-principle studies several simple 5-ALA-HPO conjugates and small dendritic 5-ALA compounds containing a HPO were synthesized. These non-toxic conjugates, that enable a simultaneous delivery into the cell, were tested in-vitro in PAM 212 keratinocytes. They also achieved an enhanced accumulation of PpIX and light-induced cell death compared to 5-ALA alone (Unpublished data: SH Battah, AJ MacRobert, RC Hider).

While in these test compounds only one HPO moiety was attached to 5-ALA esters or dendrons, increasing the number of coordinated HPOs could in principle lead to an even stronger PpIX fluorescence.

Delivery of anticancer drugs by dendrimers and use in PDT

Due to their complex and well-defined structural and sterical organization, dendrimers possess an increased ability to target malignant tissue and are therefore favored molecules for the use in cancer treatment. The effect that describes this phenomenon is called “*enhanced permeation and retention (EPR)-effect*”. Tumor cells are rapidly proliferating and dependent on the provision of nutrition from the surrounding blood vessels. Thus, the production of the vessels is stimulated. But, compared to the vasculature of normal tissue, their endothelial cells are defective and possess wider fenestrations and increased permeability. The lack of surrounding lymphatic drainage also contributes to the effect of accumulating macromolecules like dendrimers within the tumour.

2.2.1 Bidentate iron(III)-selective chelators: 3-hydroxypyridin-4-ones (HPOs)

When a ligand like 3-hydroxypyridin-4-one (HPO, Formula 3) possesses two donor atoms, it is classified as a bidentate iron chelator. Fe^{3+} is optimally coordinated once it is chelated to the metal centre by six donor atoms in an octahedral manner. Therefore, three HPOs form a complex together with one centred iron atom (Figure 8). The metal is coordinated by the chelators' two vicinal oxygen atoms^[32].

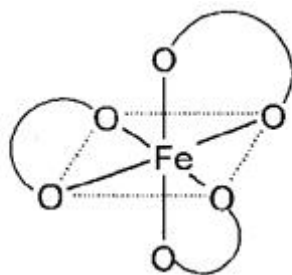


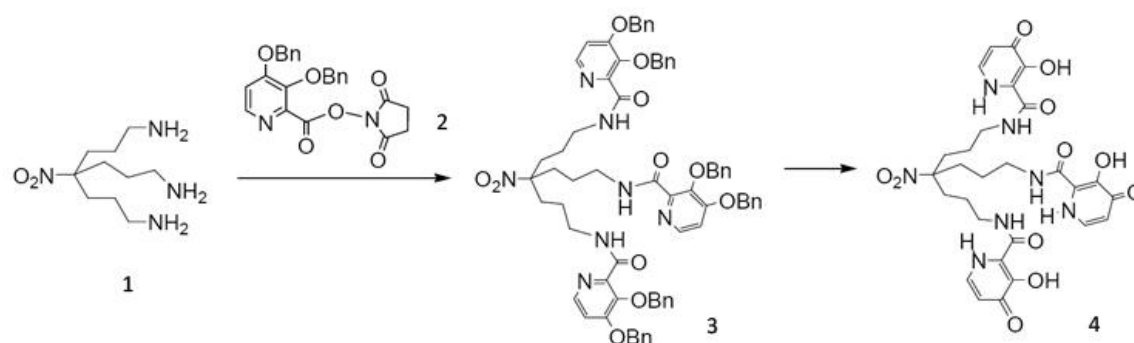
Figure 8: Schematic representation of a chelate ring formation. Three bidentate ligands (HPO) form an octahedral complex with ferric iron

One of the advantages of these Fe^{3+} -selective ligands is their ability to chelate both ferrous and ferric iron under aerobic conditions. It has furthermore been shown that “high-affinity” Fe^{3+} ligands are capable of chelating ferrous iron and alleviate the metal’s autoxidation to ferric iron^[33]. These circumstances explain why HPO-derivatives are able to inhibit the mitochondrial enzyme ferrochelatase, even though it utilizes ferrous iron for the insertion into its substrate PpIX.

2.2.2 Synthesis of iron binding dendrimers

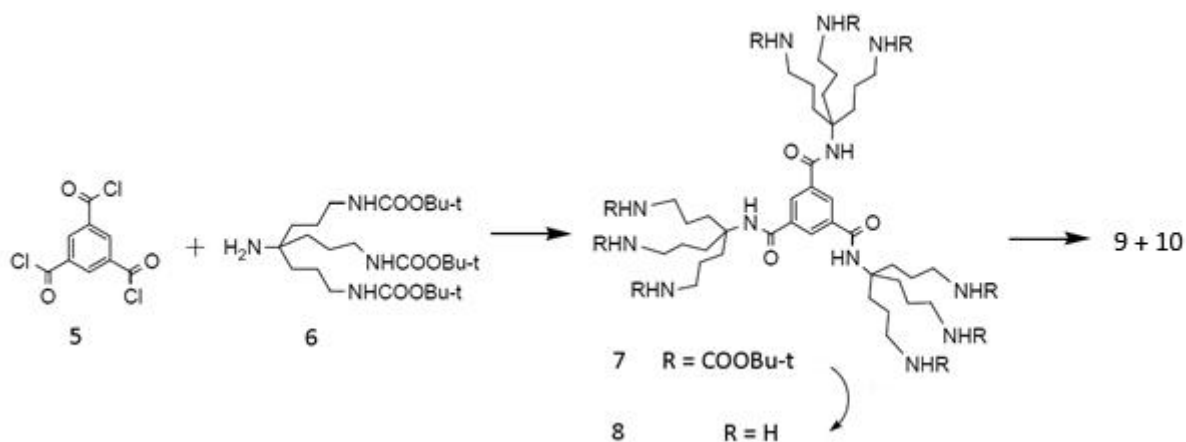
Over the past five years, a wide range of iron-chelating dendrimers has been synthesized at King’s College London^{[34] [35] [36]}. They have been prepared by both divergent and convergent routes and incorporate HPO-derivatives as iron chelating agents. The bidentate HPOs on the dendrimer surface range from three to 27 and are attached to the branching units via amide linkers.

Zhou et al.^[36] synthesized a range of iron(III) binding dendrimers for the potential use in a method of preventing iron overload. Dendritic chelators possess a high molecular weight and are not absorbed by the intestine. They are able to bind dietary iron and form nontoxic complexes that can be easily excreted in the feces. In a first synthetic route (Scheme 1) the hexadentate ligand **4** was synthesized by coupling three bidentate terminal HPO-ligands **2** to a tripodal triamine **1**. The activated bidentate HPO end-groups **2** were prepared from maltol in eight steps.



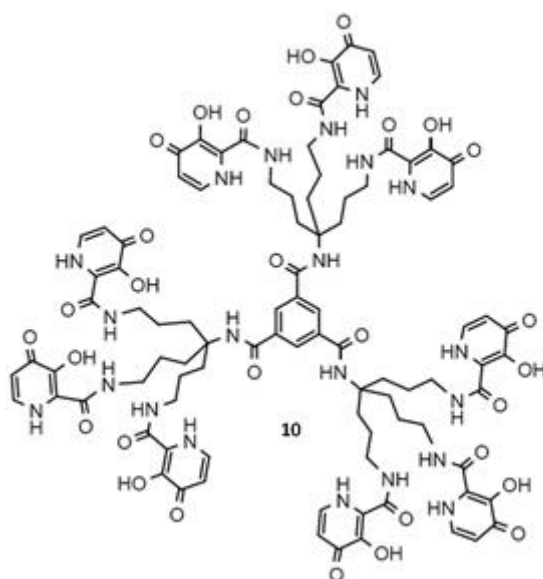
Scheme 1: Synthesis of a hexadentate chelator^[35]

In a next synthetic route three of the previously described hexadentate ligands were combined in one dendritic chelator **10** (Formula 4), that possesses an extremely high affinity for iron(III). Employing the divergent synthetic route, the synthesis started off with the coupling of three ester-protected tripodal branching units **6** to a benzene tricarbonyl trichloride core **5**. The resulting ester-protected compound **7** was treated with formic acid and diluted base to afford the deprotected molecule with the free amine groups **8**.



Scheme 2: Synthesis of a dendritic chelator using a multifunctional core, a tripodal branching unit and bidentate HPO-derivatives

This step was followed by the amide formation with the activated pyridinone **2** (Scheme 1). The resulting first generation dendrimer **10** is shown in formula 4 after deprotection of the benzyl-protected dendrimer **9** (not displayed here). It contains three tripodal branching units attached to a multifunctional core and it is terminated by nine iron chelating HPO-derivatives.

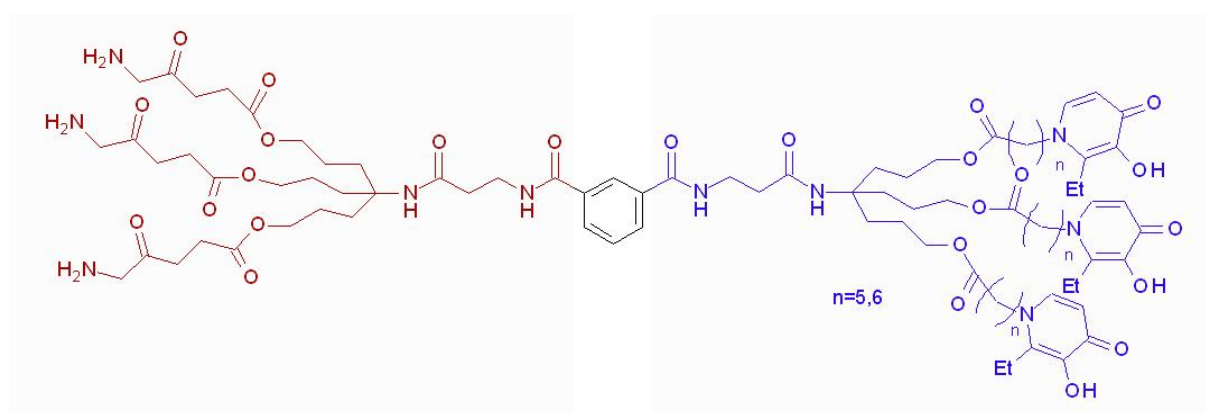


Formula 4: Hexadentate dendritic chelator based on a benzene tricarboxyl core polyamine dendrimer^[35]

3 Aim of the project and synthetic schemes

The aim of my four month project at King's College London (KCL) was to gain more knowledge about organic chemistry and to synthesize one half of a first generation dendrimer (Formula 5) that might be used in photodynamic therapy. Dr. Tao Zhou is the main synthetic chemist in this project at KCL and has been working on dendrimeric chelators over the past five years^[34-36]. Throughout the first weeks of my stay he was guiding me through the first synthetic attempts and I gradually became more acquainted with the working environment of this laboratory. During this time and also after Dr. Zhou's departure to China I was advised by Professor Robert C. Hider, the lead scientist in the Medicinal Chemistry Group at KCL.

The synthesis and the biological studies of these dendritic conjugates of the synergistically acting drugs 5-ALA and HPO are part of a collaborating project between the University of Essex and KCL. The left part of the first generation dendrimer in Formula 5, colored red, contains the 5-ALA moieties and is synthesized in Essex by the group of Dr. Sinan Battah^[31, 37]. The other half of the dendrimer, colored blue (Formula 5), possesses HPO-derivatives as terminal groups. Both of these separate building blocks, or dendrons, are attached to a bi-functional core through a linking unit, -alanine. The linkers are coupled to a tripodal branching unit via amide bonds, whereas the three HPO-derivatives and accordingly the three 5-ALA molecules are connected via ester bonds. The two dendrons will be assembled and biologically tested at the University of Essex.

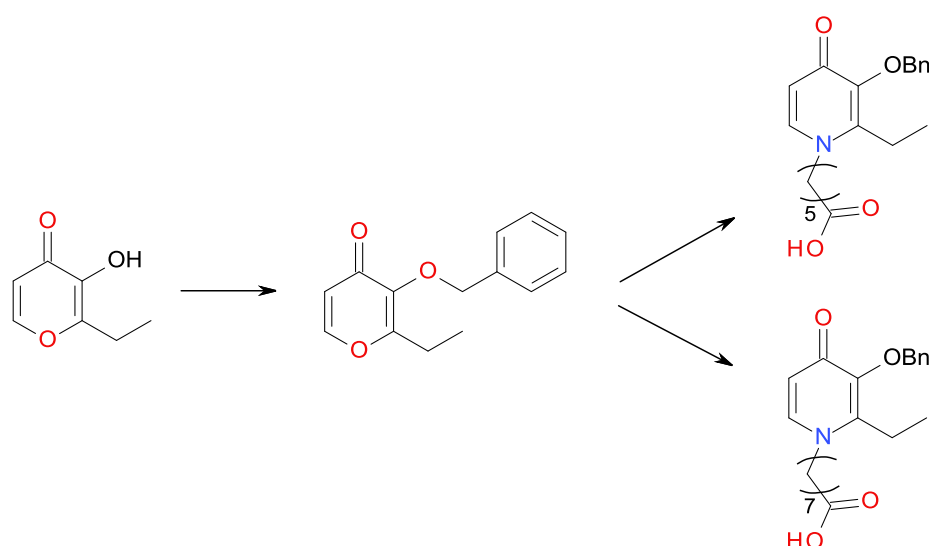


Formula 5: First generation dendrimer for photodynamic therapy containing both 5-ALA and HPO-derivative moieties

3.1 Synthesis of the HPO-derivatives

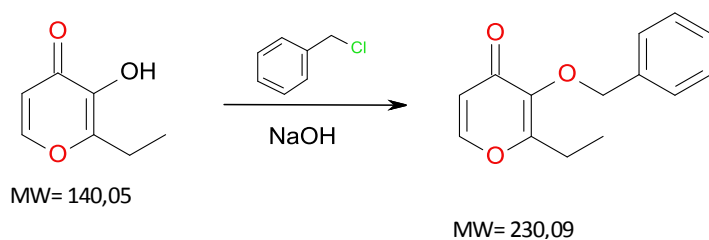
The previously presented first generation dendrimer contains three terminating HPO-derivatives with aliphatic alkyl spacers between the HPO rings and the ester groupings of the branching unit. Biological tests of single conjugates of 5-ALA and HPO with several different chain lengths of alkyl, polyethyleneglycol and polyethyleneamine spacers revealed the most suitable spacers for the incorporation within the dendrimer. It was found that a carbon chain length of either six or eight is most appropriate in terms of lipophilicity and optimized cellular uptake. (Unpublished data: SH Battah, AJ MacRobert, RC Hider)

In Scheme 3 it is shown how two HPO derivatives with a different length of carbon atoms in their aliphatic chain are prepared from benzyl-protected ethyl maltol.



Scheme 3: Overall scheme for the synthesis of two different HPO derivatives, using ethyl maltol as a starting material

3.1.1 Synthesis of 3-benzyloxy-2-ethyl-pyran-4-one



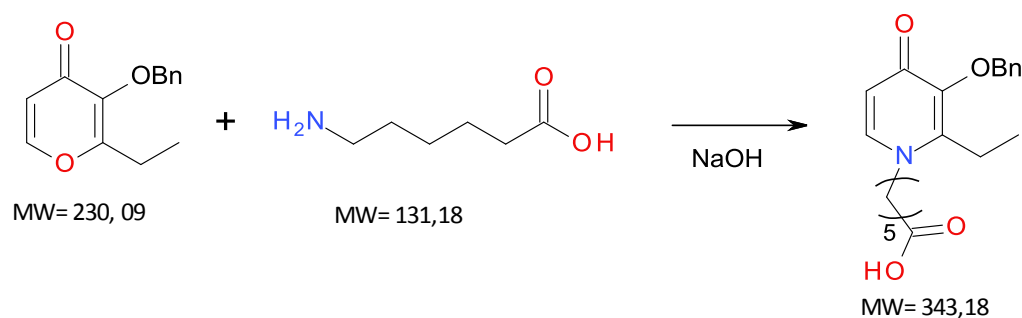
Scheme 4: Benzylation of ethyl maltol

The starting material for the preparation of the two different HPO-derivatives was 2-ethyl-3-hydroxy-pyran-4-one (ethyl maltol). First it was necessary to protect the hydroxy group of ethyl maltol by benzylation (Scheme 4). Applying the Williamson ether synthesis method, the starting material was initially treated with sodium hydroxide in order to generate the nucleophilic alkoxide. The alkyl halide benzyl chloride was added dropwise and refluxed overnight. The nucleophilic alkoxide ion could therefore attack the electrophilic carbon atom of the benzyl chloride via an $\text{S}_{\text{N}}2$ reaction and form the desired benzyl ether. The solvent was removed, the residue was dissolved in dichloromethane (DCM) and washed with 5% sodium hydroxide (NaOH), brine and finally with water. Diethyl ether was added to the concentrated residue and achieved precipitation of slightly yellow crystals when the solution was allowed to stand in the fridge overnight. The crystals, which were obtained in an acceptable yield, were washed with hexane/diethyl ether and used for the following step.

The results from the ^1H - nuclear magnetic resonance (^1H -NMR) investigation clearly proved the presence of the protective benzyl group. The hydrogen atoms from the CH_2 -group of the benzyl group appeared as a singlet at a chemical shift of 5,10 parts per million (ppm), whereas the aromatic hydrogen atoms could be found as a multiplet at δ 7,24 – 7,33 ppm.

The detailed synthetic procedure is described in section 5.1.

3.1.2 Synthesis of 3-benzyloxy-1-carboxypentyl-2-ethyl-4(1H)-pyridinone



Scheme 5: Synthesis of 3-benzyloxy-1-carboxypentyl-2-ethyl-4(1H)-pyridinone

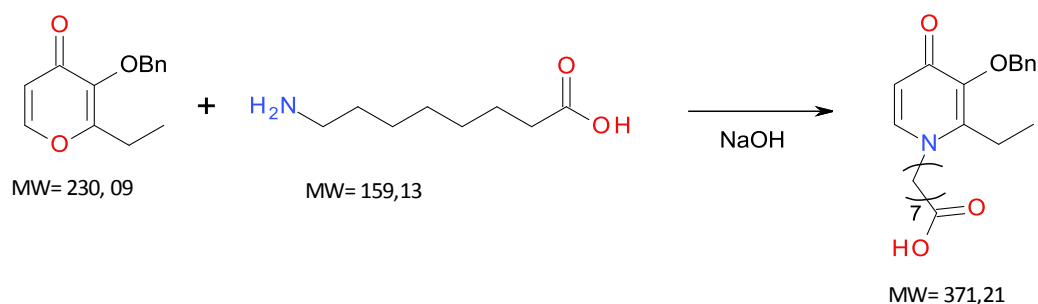
The conversion of the previously prepared benzyl-protected ethyl maltol into a pyridinone-derivative is an amine insertion reaction. The mechanism that is accepted for this type of reaction involves the nucleophilic attack of the 4-pyrone by a primary amine. The nucleophilic amine targets the oxygen in the ring of the 4-pyranone, the ring is therefore opened and a molecule of water is eliminated. Subsequently, the ring closes again and affords the desired 4-pyridinone^[38].

As shown in (Scheme 5), 2-ethyl-3-benzyloxy-4-pyranone was refluxed overnight with the primary amine 6-aminohexanoic acid (aminocaproic acid) after the pH-value had been adjusted to 13 with NaOH. Once the reaction had been completed, the solution was acidified (pH 4) to achieve the precipitation of an oily mass. This oily precipitate was extracted from the aqueous phase with DCM and the organic phase was concentrated in vacuo. The residue was dissolved in the smallest possible amount of ethanol (EtOH) and then diethyl ether was added slowly until the appearance of a slight cloudiness. When the solution was stored in the fridge overnight, crystals formed in an acceptable yield and the desired HPO-derivative with a carboxypentyl-substitution on the ring nitrogen atom had been successfully achieved.

The structure was determined by ¹H-NMR and the two characteristic olefinic protons at position 5 and 6 of the pyridinone ring were observed at δ 6,69 and 7,42 ppm. The carboxylic proton was observed at δ 9,96 ppm.

The detailed synthetic procedure is described in section 5.2.

3.1.3 Synthesis of 3-benzyloxy-1-carboxyheptyl-2-ethyl-4(1H)-pyridinone



Scheme 6: Synthesis of 3-benzyloxy-1-carboxyheptyl-2-ethyl-4(1H)-pyridinone

The preparation of the analogue HPO derivative with a carboxyheptyl substitute at the ring nitrogen atom (Scheme 6) was initially carried out in exactly the same way as the previously described 3-benzyloxy-1-carboxypentyl-2-ethyl-4(1H)-pyridinone.

In this approach, it was more difficult than expected to obtain crystals in a good yield. The starting conditions for this reaction with 8-aminooctanoic acid were the same as the previously described reaction with aminocaproic acid. Once the reaction had finished and the pH-value of the aqueous solution lowered to 4, an oily mass precipitated from the water phase. After having extracted this oil with an organic solvent and concentrated it in vacuo, the residue was dissolved in a small amount of EtOH and diethyl ether was added to initiate precipitation of crystals. Several ratios of EtOH/diethyl ether and other solvents were investigated to induce the formation of crystals, but in most cases the phenomenon of “oiling out” occurred or the yield of the crystals was low (around 25%).

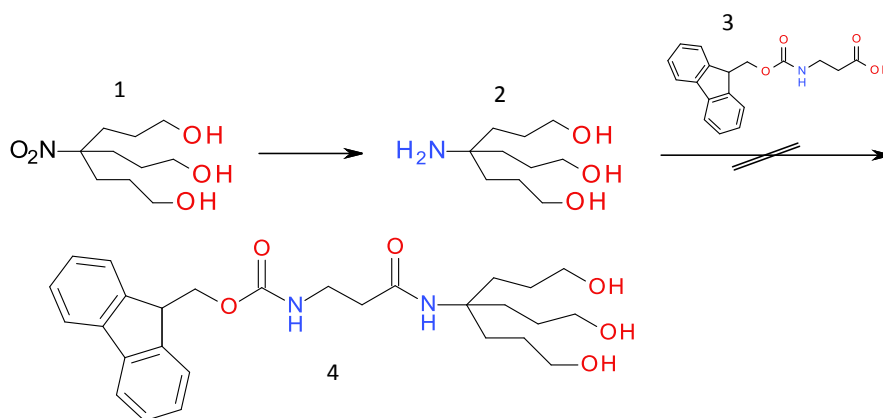
The literature was searched for ways of improving the yield of the compound and it was found that Katoh et al.^[39] synthesized a similar HPO derivative in 2003 bearing a methyl group instead of the ethyl group in position 2. Following these instructions and modifying them slightly resulted in an acceptable high yield of crystals. The reaction was left to reflux for 48 hours instead of 24 hours and the aqueous solution was now acidified in two steps: firstly hydrochloric acid (HCl) was added until pH 8 was reached, followed by extraction with DCM to remove the remaining starting material (2-ethyl-3-benzyloxy-pyran-4-one). In the second step the solution was acidified with citric acid until pH 4 was reached and the desired compound precipitated out of the water phase as pale white flakes.

The structure was determined by $^1\text{H-NMR}$. The two characteristic olefinic protons at position 5 and 6 of the pyridinone ring were observed at δ 6,57 and 7,34 ppm. The protons in the CH_2 groups of the carbon chain deriving from aminooctanoic acid could be easily assigned and distinguished from the signals of the protons in the HPO derivative with the attached aminocaproic acid.

The detailed synthetic procedure is described in section 5.3

3.2 Preparation of the tripodal branching unit 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol and attempt of coupling it with the Fmoc or Boc - protected linking unit β -alanine

The scheme below (Scheme 7) shows the pathway for the synthesis of (9-methylfluoren-9-yl)methyl-N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate **4**, which consists of the fluorenylmethyloxycarbonyl (Fmoc) - protected linking unit β -alanine **3** and the tripodal branching unit that is attached via an amide bond. The starting material 4-(3-hydroxypropyl)-4-nitro-1,7-heptanediol (nitromethane-tris-propanol) **1** was successfully reduced in two different approaches to yield in crude 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol (bis-homotris^[40]) **2**. It was tried to attach the crude bis-homotris to Fmoc- β -alanine through amide bond formation, but all of these attempts were unsuccessful.



Scheme 7: Overall scheme for the synthesis of (9-methylfluoren-9-yl)methyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate (**4**)

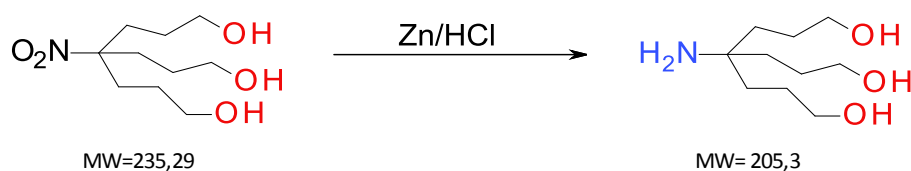
In another approach commercially purchased, pure bis-homotris was used for the coupling reaction with *tert*-butyloxycarbonyl (Boc) - protected β -alanine. Again, the coupling was not

successful even when different conditions and reagents were applied. This reaction is not shown in Scheme 7, but will be discussed in section 3.2.3.

3.2.1 Synthesis of 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol (Bis-homotris)

Bis-homotris has been described in the literature by Newkome et al.^[40] as “cascade building block” and is also commercially available. However, initially it was decided to synthesize this building block in order to circumvent increased costs due to the comparatively high price of the compound.

Reduction of nitromethane-tris-propanol using Zn/HCl as reducing agents



Scheme 8: Synthesis of 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol using Zn/HCl as reducing agents

In the first approach (Scheme 8), the nitro group of the commercially purchased nitromethane-tris-propanol was reduced to an amino group using Zinc (Zn) and HCl. The starting material was dissolved in EtOH and HCl was added dropwise while the zinc powder was added in small portions at room temperature. As there was no visible indication of effervescence, the temperature was increased and the mixture was refluxed until the thin layer chromatography (TLC) analysis revealed complete reaction. After the removal of the solid zinc powder, the solvent was evaporated and the residue was dissolved in ethyl acetate.

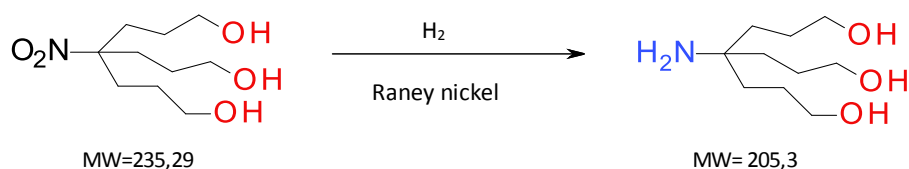
The organic phase was washed with a solution of sodium bicarbonate (NaHCO_3), whereon bubbles of gas were welling up. Upon the addition of potassium bicarbonate (K_2CO_3) the aqueous solution turned white and creamy. This was done in order to deprotonize the product and to permit partition into the organic phase. The two phases were analyzed by TLC using a solution of ninhydrin in butanol and acetic acid in order to reveal the amino groups as purple spots. It was found that the entire product was present in the aqueous phase together with the inorganic salts that caused the white cream. Therefore, the salt was filtered off and the water was removed under reduced pressure using a strong pump. The residue, white crystals in an oily mass, was dissolved in methanol (MeOH) to

separate the salt from the compound. The inorganic salt was filtered off and the solvent was evaporated again. As there was still some salt present, the residue was dissolved in anhydrous isopropanol. Filtration and removal of the solvent afforded the crude product.

Due to the highly hydrophilic character of bis-homotris, the work-up of the reaction was relatively problematic. Once the compound was present in the aqueous phase it was difficult to separate it from the inorganic salts.

The detailed synthetic procedure is described in section 5.4.1.

Reduction of Nitromethane-tris-propanol using Raney nickel as reducing agent

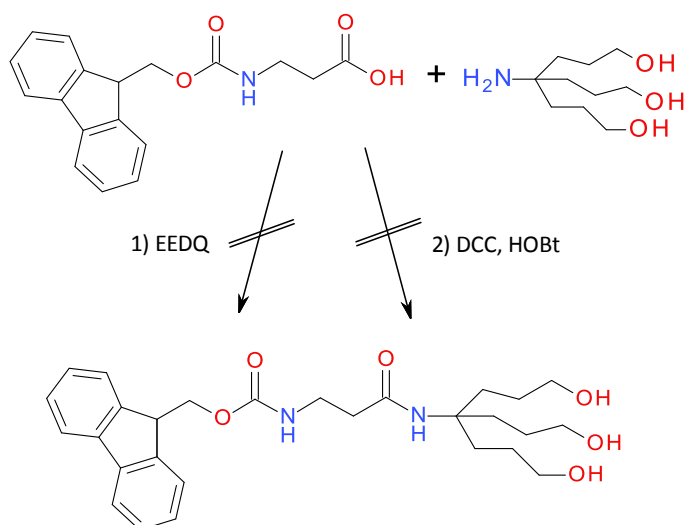


Scheme 9: Synthesis of 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol using Raney nickel as reducing agent

In order to circumvent the difficulties that occurred in the previous method, the reduction of nitromethane-tris-propanol was attempted in the absence of water, using Raney nickel. In this approach (Scheme 9) nitromethane-tris-propanol was hydrogenated for 24 hours with specially prepared T-1 Raney nickel, which had been previously made following the instructions of Domingues et al.^[41]. This reaction afforded the crude product in a good yield and seemed to be the superior choice of the two approaches for this reduction.

The detailed synthetic procedure is described in section 0.

3.2.2 Attempt to synthesize (9-methylfluoren-9-yl)methyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate



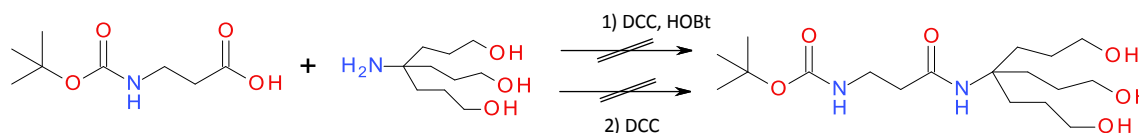
Scheme 10: Attempt to synthesize (9-methylfluoren-9-yl)methyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate in two different approaches using EEDQ (1) and DCC/HOBt (2) as coupling agents

As demonstrated in Scheme 10 pathway 1, the crude product of the previous reaction, Bis-homotris, was reacted overnight at room temperature with Fmoc- β -alanine and the widely used coupling agent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in anhydrous dimethylformamide (DMF). The results of the TLC analysis indicated the formation of new products, but it was not completely clear which one should be isolated. The white precipitate was filtered off, DMF was removed under reduced pressure and the residue was dissolved in a small amount of ethyl acetate. This solution was passed through a silica gel column eluting with ethyl acetate/hexane (1:5) to achieve a separation of the different products. Mass spectrometry (MS) analysis of the collected fractions showed that none of the isolated fractions contained the desired compound.

In pathway 2 the previously prepared Bis-homotris, Fmoc- β -alanine and the widely used peptide coupling agents N,N' -dicyclohexyl-carbodiimide (DCC) and 1-hydroxy-benzotriazole hydrate (HOBt) were reacted in anhydrous DMF and left to stir overnight at room temperature. Upon removal of the white precipitate, dicyclohexylurea (DCU), the solvent was removed and a yellow sticky residue was obtained. Analyzing a sample of this residue by MS demonstrated the absence of the desired.

The same reaction was also carried out with DCC alone and led thus to a similar result where no traces of the desired compound were detected on MS analysis.

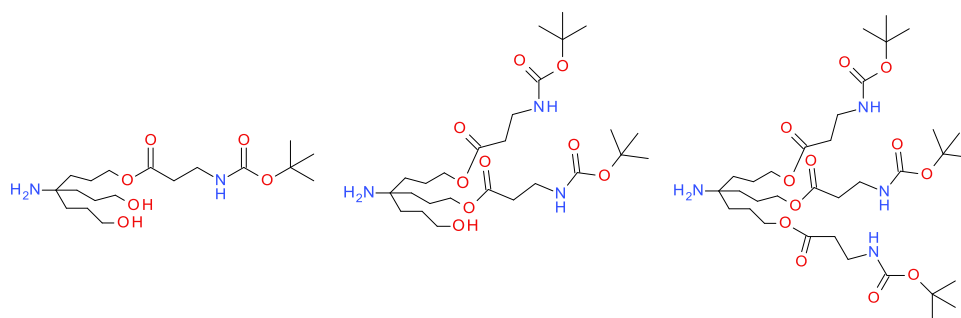
3.2.3 Attempt to synthesize tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate



Scheme 11: Attempt to synthesize tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate using DCC and HOBT (1) and DCC only (2) as coupling agent

In the synthetic procedures from section 3.5.2 onwards, a Boc-group protected linking unit is used instead of the initially applied Fmoc protective group. The reasons for this will be explained in section 3.4 in more detail. While experimenting with this new protective group, another attempt was carried out to couple Bis-homotris directly to the Boc - protected β -alanine linking unit. Moreover, this time commercially purchased, pure bis-homotris was used as a starting material for the reaction to investigate if the same problems occur with this material.

Pathway 1 (Scheme 11) was carried out overnight at room temperature, using the coupling agents DCC and HOBT in anhydrous DMF. The instant formation of white precipitate (DCU) indicated that some kind of reaction had occurred, which was also visible on the TLC plate. Once the precipitate had been filtered off and the solvent had been removed, a sample of the residue was used for a MS-analysis to receive further information about the outcome of the reaction. The MS data showed peaks at the molecular weight of each of the three esters (Formula 6) and not of desired amide.



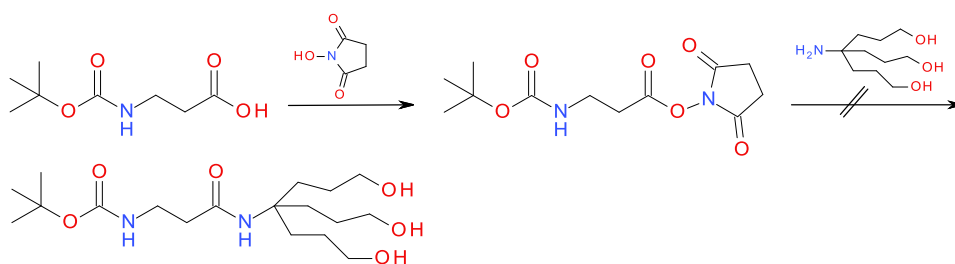
Formula 6: Formation of the three esters instead of the amide

This is rather surprising, considering that amino groups are generally stronger nucleophiles than hydroxyl groups and should therefore attack the DCC - activated ester of Boc- β -alanine more rapidly^[42].

3.3 Synthesis of the N-hydroxysuccinimide ester of the linking unit Boc- β -alanine and attempts to couple it with bis-homotris

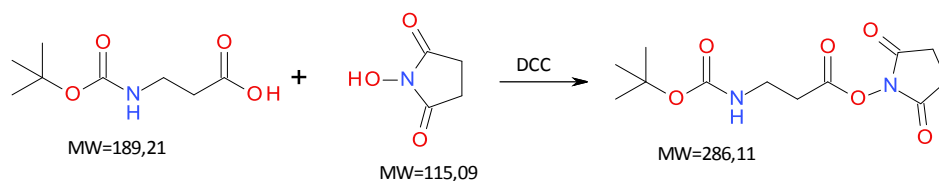
Earlier attempts to create an amide bond between the linking unit and the unprotected bis-homotris using either DCC and HOBt or DCC only as coupling agents failed. This is probably due to the decreased nucleophilic character of the amino group in bis-homotris which is most possibly caused by the sterical influence of the hydroxyl groups forming multiple intramolecular hydrogen bonds. In a different approach (Scheme 12), the active N-hydroxysuccinimide ester of Boc- β -alanine was synthesized and then it was tried to let it react specifically with the amino group of bis-homotris. The preparation of N-hydroxysuccinimide esters is a common step in peptide synthesis in order to achieve amide bond formation in the presence of hydroxyl groups. As shown below it was only possible to prepare the ester, the desired coupling failed.

After having observed all these difficulties associated with the use of the unprotected bis-homotris, different approaches with protected derivatives were tried.



Scheme 12: Overall scheme for the synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate

3.3.1 Synthesis of the N-hydroxysuccinimide ester of Boc- β -alanine (((2,5-dioxopyrrolidin-1-yl) 3-(tert-butoxycarbonylamino)propanoate)



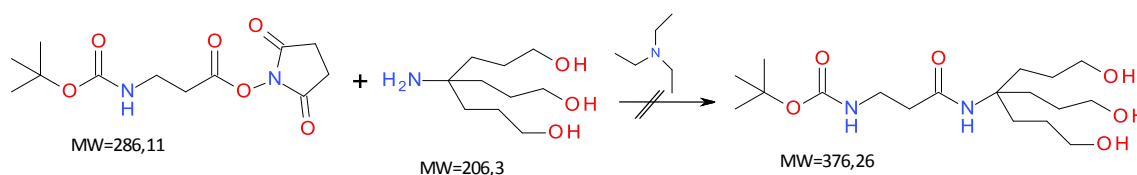
Scheme 13: Synthesis of the N-hydroxysuccinimide ester of Boc- β -alanine

As demonstrated in Scheme 13, the linking unit Boc- β -alanine and N-hydroxysuccinimide, which is an activating agent for carboxylic acids, were dissolved in dry ethyl acetate and reacted with the coupling agent DCC at room temperature. The mixture was stirred at overnight and stored in the fridge for a couple of hours to remove as much DCU as possible by precipitation. DCU was filtered off and the filter cake was washed with cold ethyl acetate. The combined filtrates were concentrated and white crystals formed at room temperature in a good yield.

$^1\text{H-NMR}$ analysis supported the presence of the obtained N-OH-succinimide ester. The two CH_2 groups adjacent to the carbonyl groups in the succinimide appear together with the $-\text{CH}_2\text{-COO-N}<$ signal at 2,76-2,78 ppm. The infrared (IR) spectrum shows a sharp N-H stretching band at 3362 cm^{-1} deriving from the nitrogen next to the Boc-group. The C=O stretching bands from the cyclic imide ring appear at 1779 and at around 1700 cm^{-1} . An amide I band appears at 1688 cm^{-1} , whereas the amide II band can be found at 1523 cm^{-1} .

The detailed synthetic procedure is described in section 5.5.

3.3.2 Attempt to synthesize tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate



Scheme 14: Attempt to synthesize tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate

Scheme 14 shows the attempt of coupling the activated carboxylic acid and bis-homotris. Bis-homotris was gently heated and stirred in order to dissolve completely in anhydrous DMF and then cooled to room temperature again. The N-hydroxysuccinimide ester of Boc- β -alanine and the non-nucleophilic base triethylamine were added to the stirred solution. The mixture was stirred at room temperature and checked by TLC after about 15 min (DCM/MeOH, 8:2 + ninhydrin). Several new spots had formed on the TLC which indicated that a reaction had taken place. After about 1,5 hours of stirring, the spots of the starting material had vanished nearly completely. The clear solution was poured into ice-cold water and stirred vigorously. As no precipitate had formed, the water and the DMF were removed again under reduced pressure. The residue was slightly yellow colored oil. This residue was put on a silica-gel column for the isolation of two spots that were likely to be the desired product. DCM/MeOH (8:2) was used as an eluent for the column. It was not possible to separate the spots completely, but in that way at least a certain level of purification could be reached.

Two sets of fractions were collected, the solvent was removed and samples were given away for MS-analysis. In both cases the peaks did not correlate to the expected mass. The desired compound should have had a peak at a mass-to-charge ratio (m/z) of 376, whereas the sample-peaks appeared at m/z 741 and 570. We were unable to identify these peaks.

3.4 Possible reasons for the synthetic difficulties occurring when the unprotected bis-homotris was introduced as a branching unit

Initially, bis-homotris was utilized in various attempts as a tripodal branching to synthesize the main building block for the desired first generation dendrons. This molecule has already been described in the literature^[40], but has not been applied for the direct use in dendrimer synthesis. During this project, a few different methods have been employed to accomplish an amide bond formation between the carboxylic acid of the protected β -alanine and the amine triol bis-homotris. As described earlier, none of these attempts had been successful or satisfying due to the unusual chemical properties of this molecule.

Bis-homotris possesses hydroxyl groups and an amino group in a geometric proximity that allows the simultaneous formation of three intramolecular hydrogen bonds. It is likely that

the lone pair of the nitrogen in the amine group is attracted by the hydrogen atom in one of the hydroxyl groups and therefore forms an intramolecular hydrogen bond. Additionally, the two hydrogen atoms of the primary amine in bis-homotris are able to act as hydrogen bond donors for the oxygen lone pairs of the remaining two hydroxyl groups. If all the possible intramolecular hydrogen bonds are formed, the molecule is arranged in a highly symmetrical conformation. In the three dimensional picture of bis-homotris that is presented below (Figure 9), an energy minimizing performance visualized this interesting arrangement of intramolecular hydrogen bonds. This model was generated with the program ChemBio3D.

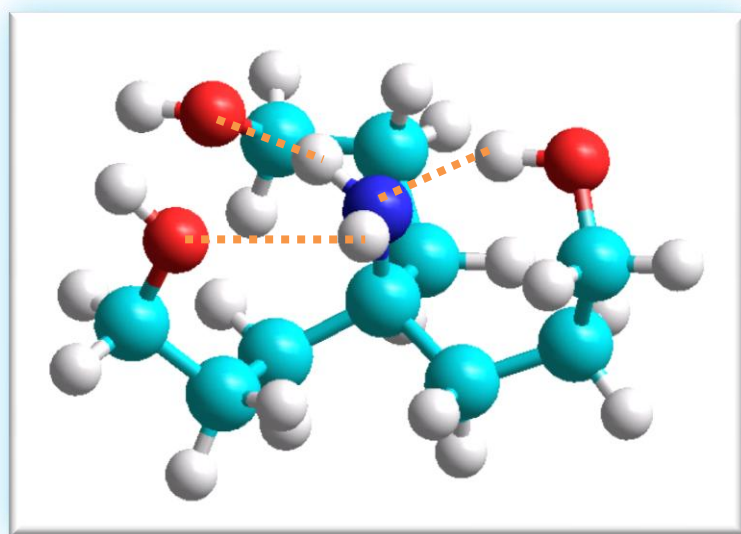


Figure 9: Three dimensional model of an energy-minimized bis-homotris using ChemBio3D

Assisted by Dr. XL Kong, the distances for these hydrogen bonds have been determined using the program mentioned above. The calculated lengths for the three presumably formed hydrogen bonds are stated in Å (angstrom) as follows:

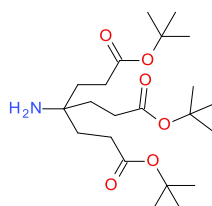
- N---HO 1,70 Å
- NH---O 2,39 Å
- NH---O 2,41 Å

These three possible intramolecular hydrogen bonds and the steric conformation of the hydroxyl chains contribute to a decreased availability of the amino group during chemical

reactions. The three dimensional model also explains that the compound was not readily dissolving in various solvents. It is assumed that the closed, intramolecular hydrogen bond ring system predominates under conditions of chemical reactions.

To circumvent these complications, protected derivatives were used in further synthetic schemes.

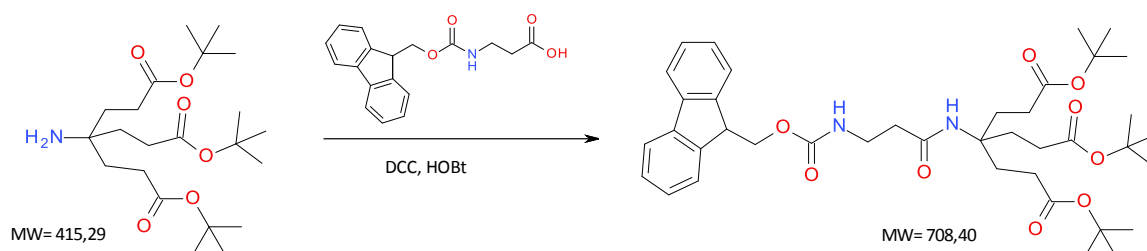
3.5 Investigation of ditert-butyl 4-amino-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate as the tripodal branching unit



Formula 7: New building block ditert-butyl 4-amino-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate

This new building block reagent (Formula 7) was provided by Tao Zhou who had prepared it following the procedure described by Newkome et al.^[43]. Using the protected branching unit instead of the triol bis-homotris should help to circumvent the problem of a decreased availability of the amine group.

3.5.1 Coupling of a *tert*-butyl ester - protected tripodal branching unit with the Fmoc - protected linking unit β -alanine followed by an attempt to reduce the protective *tert*-butyl esters



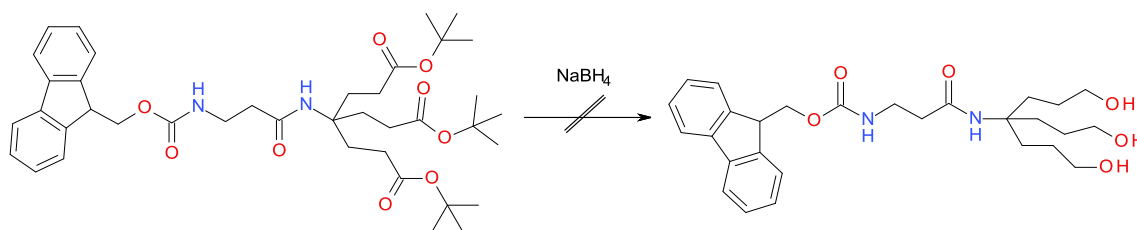
Scheme 15: Synthesis of ditert-butyl 4-(3-tert-butoxy-3-oxo-propyl)-4-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propanoylamino]heptanedioate using DCC and HOBt as coupling agents

Applying peptide coupling procedures, Fmoc- β -alanine was treated with the amino ester, DCC and HOBt in anhydrous DMF and stirred at room temperature overnight. The precipitate was filtered off and the solvent was removed in vacuo to furnish orange colored oil. This

residue was passed through a silica gel column and white crystals were obtained as the desired compound (ditert-butyl 4-(3-tert-butoxy-3-oxo-propyl)-4-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propanoylamino]heptanedioate) in an excellent yield.

MS analysis confirmed the presence of the coupling product with a peak at m/z 731,20 ($[M+Na]^+$). 1H -NMR investigation lend further support for the correct compound when peaks deriving from the aromatic Fmoc group appeared at a range of 7,23-7,70 ppm. Additionally, the methyl groups were found as singlets at 1,36 and 1,37 ppm. The IR spectrum of Boc- β -Ala-OH was compared to the IR spectrum of the obtained compound and instead of one sharp N-H stretch at 3442 cm^{-1} , two sharp N-H stretches could be observed at 3371 and 3313 cm^{-1} .

The detailed synthetic procedure is described in section 5.6.

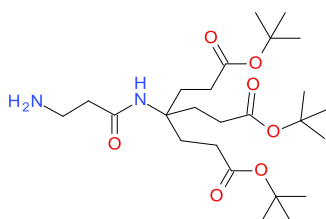


Scheme 16: Attempt to reduce the *tert*-butyl esters using sodium borohydride (NaBH_4) as reducing agent

As shown in Scheme 16, it was then attempted to convert the tertiary butyl esters into alcohols using sodium borohydride as a reducing agent. The starting material, containing the three esters, was therefore dissolved in anhydrous tetrahydrofuran (THF) and heated under reflux at 70°C for one hour while solid sodium borohydride was added in small portions. When anhydrous MeOH was added dropwise, the solution stopped bubbling and cleared up. The reaction was checked by TLC and was judged to be complete after 1,5 hours. A solution of saturated ammonium chloride was added and white precipitate formed immediately. The mixture was left to stir for about 1,5 hours. The solution was then extracted with ethyl acetate and dried over sodium sulfate. Removing the solvent afforded a white, sticky residue in a high yield.

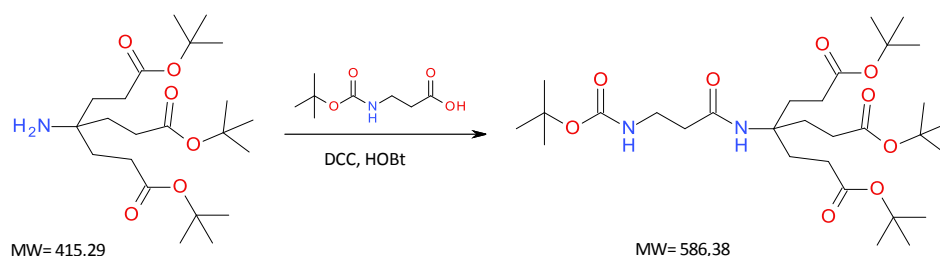
The analysis of the data of the MS and the 1H -NMR spectrum revealed that instead of the desired triol, the compound shown in Formula 8 had formed. The 1H -NMR demonstrated the loss of the aromatic part of the molecule and in the MS spectrum a parental peak appeared

at m/z 487, which corresponds to the formula below. Apparently, the Fmoc-group was not sufficiently stable and the conditions led to the decomposition of the molecule.



Formula 8: Product of the attempted reduction: Loss of the Fmoc-group

3.5.2 Coupling of a *tert*-butyl ester - protected tripodal branching unit with the Boc - protected linking unit β -alanine followed by an attempt to reduce the protective *tert*-butyl esters



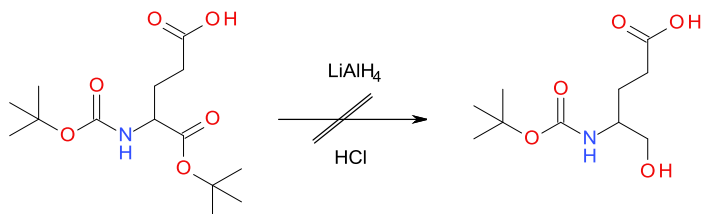
Scheme 17: Synthesis of ditert-butyl 4-[3-(tert-butoxycarbonylamino)propanoylamino]-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate using DCC and HOBt as coupling agents

This time the coupling of the branching unit and the linking unit was carried out using a Boc-protected β -alanine instead of the unstable Fmoc-group. As presented in Scheme 17, Boc- β -alanine was treated with the amino ester, DCC and HOBt in anhydrous DMF and stirred at room temperature overnight. The precipitate was filtered off and the solvent was removed in vacuo to furnish red colored oil. This residue was passed through a silica gel column and white crystals were obtained as the desired compound (ditert-butyl 4-[3-(tert-butoxycarbonylamino)propanoylamino]-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate) in a good yield.

MS analysis confirmed the presence of the coupling product with a peak at m/z 609,20 ($[M+Na]^+$). 1H -NMR analysis showed one sharp singlet at 1,38 ppm deriving from the protons of all the methyl groups. The other peaks appeared as expected and could be easily assigned. The IR spectrum of Boc- β -Ala-OH was compared to the IR spectrum of the obtained

compound and instead of one sharp N-H stretch at 3442 cm^{-1} , two sharp N-H stretches could be observed at 3389 and 3340 cm^{-1} .

The detailed synthetic procedure is described in section 5.7.



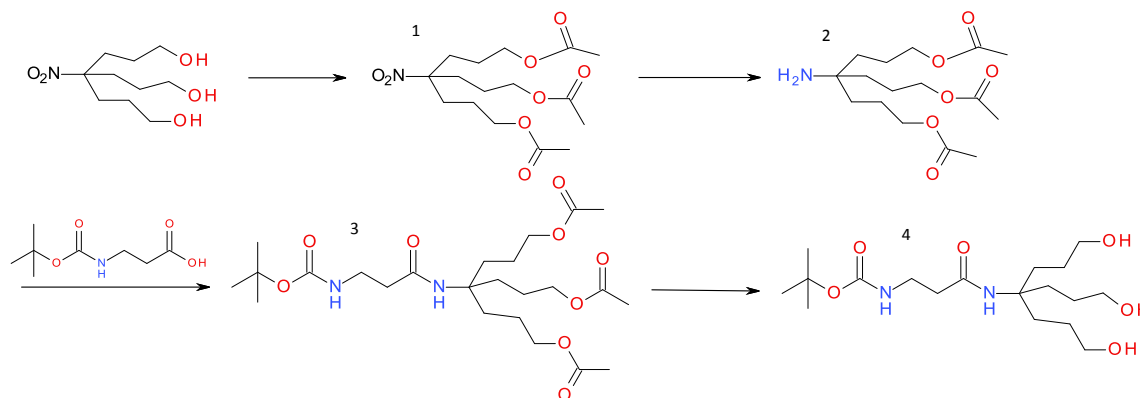
Scheme 18: Attempt to reduce the tertiary butyl ester of Boc-Glu-OtBu using lithium aluminum hydride (LiAlH_4) as reducing agent

In the next step it was decided to use Boc-Glu-OtBu as a test molecule for the selective reduction of the tertiary butyl ester. If this reaction had been successful, the product of the previous step, ditert-butyl 4-[3-(tert-butoxycarbonylamino)propanoylamino]-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate, would have been reacted under the same conditions. Both the test molecule and the “real” compound possess a Boc group protecting an amine and a tertiary butyl ester and are therefore comparable in their chemical behavior.

The reaction shown in Scheme 18 was carried out in various conditions, but no attempt led to the desired product. In the first attempt lithium aluminum hydride (1M solution in THF) was added to a solution of Boc-Glu-OtBu in anhydrous THF, stirred at 0°C for two hours and then treated with diluted HCl. The solvent (THF) was removed under reduced pressure and remaining the aqueous phase was washed with ethyl acetate. The residue of the organic phase was analyzed by ^1H -NMR and MS. The desired product had not been found, but a lot of starting material was still present.

In other attempts Boc-Glu-OtBu was added in small portions to the solution of LiAlH_4 in THF and the temperature was increased constantly until it was refluxed for one hour. Monitoring the reaction by TLC showed that a new spot had formed at the bottom of the plate. In the MS spectrum of the isolated fraction a parental peak appeared at m/z 333, which does not correspond to the desired product.

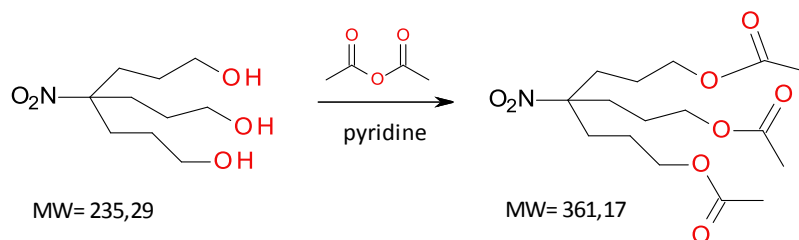
3.6 Synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate in four steps using [7-acetoxy-4-(3-acetoxypropyl)-4-amino-heptyl] acetate as the tripodal building block



Scheme 19: Overall scheme for the synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate in four steps

For the reasons explained earlier it was decided not to proceed with the unprotected bis-homotris, but to use the easily obtained triacetate of nitromethane-tris-propanol, [7-acetoxy-4-(3-acetoxypropyl)-4-nitro-heptyl] acetate **1**, as presented in Scheme 19. Catalytical reduction afforded the corresponding amine **2**, which was then easily coupled to Boc-β-alanine. The resulting compound, [7-acetoxy-4-(3-acetoxypropyl)-4-[3-(tert-butoxycarbonylamino)propanoylamino]heptyl] acetate **3** was converted into the triol **4** through a high-yielded transesterification. In this way it was possible to prepare the building block for the desired HPO containing dendron in four steps with a high overall yield. The preparation of the new building block [7-acetoxy-4-(3-acetoxypropyl)-4-amino-heptyl] acetate **2** had been prepared following the procedures described by Newkome et al.^[43]

3.6.1 Protection of nitromethane-tris-propanol through the formation of a triacetate: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-nitro-heptyl] acetate



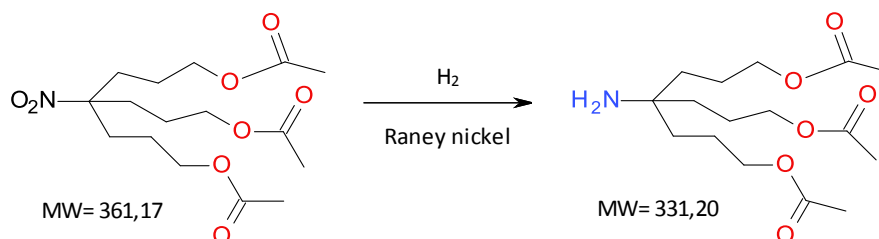
Scheme 20: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-nitro-heptyl] acetate with acetic anhydride in pyridine

In the first step, as shown in Scheme 20, the starting material nitromethane-tris-propanol was suspended in acetic anhydride and was stirred at 70°C together with pyridine for 12 hours. The reaction was judged to be complete when the TLC analysis showed all the ninhydrin-sensitive starting material to have disappeared. A new spot that could be detected with iodine dye had formed and indicated a clean reaction. The reaction solution was allowed to cool to room temperature and quenched by stirring it with water. After the neutralization with solid sodium hydrogen carbonate and extraction with diethyl ether the organic phase was washed with brine. The solvent and pyridine were removed under reduced pressure and the triacetate was obtained in a good yield as yellow oil.

Along with MS analysis, the structure was also determined by $^1\text{H-NMR}$ analysis. The transformation of the triol into the triacetate was confirmed by the presence of a singlet peak at δ 2 ppm that corresponds to the 9 protons of the three acetate methyl groups.

The detailed synthetic procedure is described in section 5.8.

3.6.2 Reduction of the nitro group: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-amino-heptyl] acetate



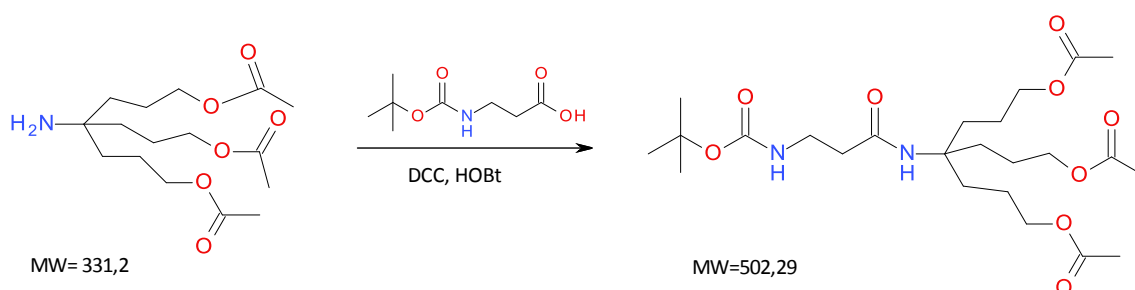
Scheme 21: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-amino-heptyl] acetate using Raney nickel as reducing agent

For the reduction of the nitro group (Scheme 21), the commercially available catalyst Raney nickel slurry in water was washed with EtOH and then added to a solution of [7-acetoxy-4-(3-acetoxypropyl)-4-nitro-heptyl] acetate in EtOH. The mixture was hydrogenated at 40 pound-force per square inch (psi). When the hydrogen uptake ceased after approximately 24 hours, the Raney nickel was carefully filtered off and the solvent was removed in vacuo. Slightly yellow oil was obtained and a brightly red, ninhydrin-sensitive spot on the TLC was then isolated by silica gel column chromatography. As a residue, slightly yellow coloured oil was obtained in a good yield.

The transformation of the nitro group into the amino group was confirmed by a $^1\text{H-NMR}$ spectrum. In the $^1\text{H-NMR}$ data for the starting material, the 6 protons of the methylene groups adjacent to the quaternary carbon atom showed a signal at δ 1,91-1,95 ppm, whereas in the amino-compound an upfield shift of these protons to δ 1,29-1,34 ppm could be observed. This is due to the fact, that the new amino group is less electronegative and has a less deshielding effect on the surrounding atoms. Furthermore the identity was proven also by MS analysis and infrared (IR) investigation. In the IR spectrum, the N-H stretches of the primary amine could be observed at a broad band around 3368 cm^{-1} , whereas the C=O stretching bands appeared at 1736 cm^{-1} . Bands for N-O stretches could not be found and supported the presence of the amino group in the molecule.

The detailed synthetic procedure is described in section 5.9.

3.6.3 Amide coupling with Boc- β -alanine: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-[3-(tert-butoxycarbonylamino)propanoylamino]heptyl] acetate



Scheme 22: Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-[3-(tert-butoxycarbonylamino)propanoylamino]heptyl] acetate using the coupling agents DCC and HOBT

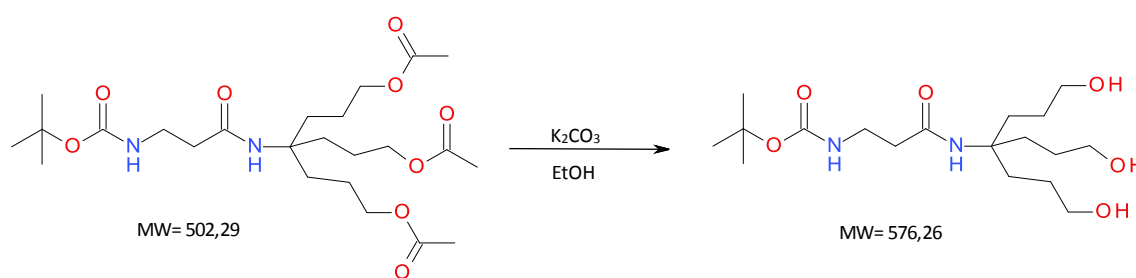
Boc- β -alanine and the previously synthesized amine (Scheme 22) were treated with DCC and HOBT in anhydrous DMF at 25 °C for approximately 24 hours. After the filtration of DCU and the removal of the solvent, the sticky residue was dissolved in DCM and subsequently washed with HCl, water and NaHCO₃. The HCl helped to get rid of the remaining HOBT while washing the organic phase with water and NaHCO₃ realized a further removal of ninhydrin-sensitive impurities with retention factors below the suspected compound-spot. Passing the concentrated residue of the organic phase through a silica gel column afforded the isolation of the desired spot on the TLC and white crystals were obtained in a very good yield.

The identity of this compound was confirmed by MS analysis, IR analysis and also by the investigation of the ¹H-NMR spectrum. The absorptions of all the protons in the molecule could be easily assigned. There was a sharp peak for the CH₃ groups of the protective Boc-group (1,36 ppm) as well as for the methyl groups of the three acetates (1,98 ppm). In the IR spectrum it was possible to see the a sharp N-H stretching band deriving from the amide at 3287 cm⁻¹ and the C=O stretching bands from both the carbamate and the amide at 1641 cm⁻¹ and 1684 cm⁻¹.

The detailed synthetic procedure is described in section 5.10.

3.6.4 Saponification of the triacetate:

Synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate



Scheme 23: Transesterification in EtOH: synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate

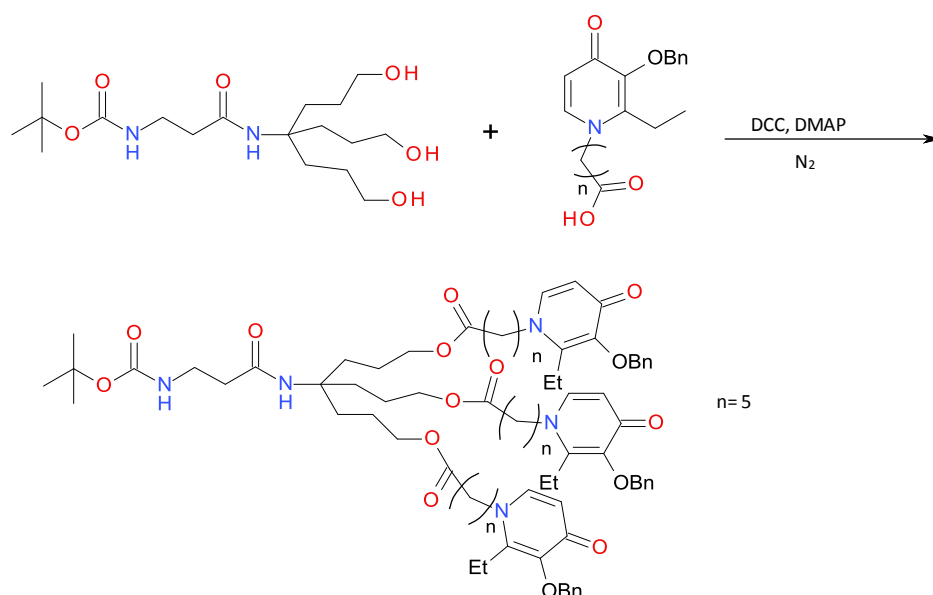
Scheme 23 demonstrates the conversion of the triacetate into the corresponding triol via transesterification in EtOH. The triacetate was dissolved in EtOH and stirred with potassium carbonate at 30 °C for 18 hours. TLC analysis revealed that all the iodine sensitive starting

material had disappeared and that a new, both iodine and ninhydrin sensitive spot had appeared at the bottom of the TLC plate. Upon removal of the fine precipitate that had formed during the reaction, the solution was concentrated in vacuo and the triol was obtained quantitatively as viscous, white oil. The triol possesses extremely hygroscopic characteristics and had to be stored in a high vacuum desiccator for a few days to solidify completely.

Having determined the structure by $^1\text{H-NMR}$ analysis, MS and IR analysis lend further support for the identity of the desired compound. In the $^1\text{H-NMR}$ data the loss of the acetate signals could be observed together with an upfield shift of the CH_2OH triplet to 3,32-3,33 ppm (from 3,95-3,98 ppm for CH_2OAc). The IR spectrum showed an intense, broad O-H stretching band from 3500 to 3300 cm^{-1} and the lack of a sharp C=O absorption stretching band which had appeared at 1738 cm^{-1} in the triacetate.

The detailed synthetic procedure is described in section 5.11.

3.7 Attempt to complete the dendron by coupling one type of HPO-derivative to the successfully established triol-branching unit



Scheme 24: Attempt of coupling the HPO derivatives to the triol using DCC and DMAP under N_2 atmosphere

For the last step of the completion of the desired dendron it was necessary to couple one type of the previously synthesized HPO-derivatives to the triol-branching unit (prepared according to section 3.6.4.).

As shown in Scheme 24, 3-benzyloxy-1-carboxypentyl-2-ethyl-4(1H)-pyridinone and DCC were dissolved in DCM/DMF (1:1) under a nitrogen atmosphere and stirred for 30 min at room temperature. At this point it was already possible to observe DCU that had precipitated from the solution. A precipitation of DCU before the addition of the triol therefore indicated an undesired side reaction. The triol, which had been dissolved in DMF, was added dropwise with a syringe over a period of another 30 min. DMAP (dissolved in DMF) was added with a syringe and the solution was left to stir at room temperature for 24 hours and then allowed to cool down in the fridge. DCU was filtered off and the solvent was removed in vacuo.

TLC analysis (9 DCM: 1MeOH) revealed a new spot that was visible under UV-light and sensitive for both ninhydrin and iodine dye. This indicated the presence of the desired compound and suggested a successful reaction.

The residue of this reaction was put on a column and eluted with DCM/MeOH (9:1). The separation of the desired spot from other impurities could not be fully achieved and it was also found that the original spot had split in three individual and very close spots.

A sample of the fractions containing these close spots had been analyzed by electrospray ionization (ESI)-MS. In the spectrum displayed in Figure 10 it was found that there were traces of the desired compound present at m/z 1352,83 $[M+H]^+$ together with incompletely coupled dendrons at m/z 702,50 and m/z 1027,67.

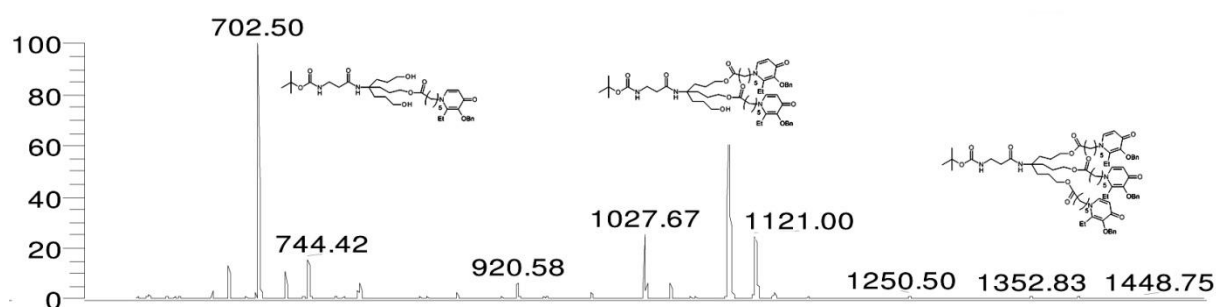


Figure 10: ESI-MS analysis of the described reaction: presence of the desired compound and incomplete coupling products; x-axis: m/z , y-axis: relative abundance

4 Instrumental details and equipment

NMR

Nuclear magnetic resonance spectra were recorded using a Bruker 400 (400 MHz) NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (Me_4Si , $\delta=0$). The resonance patterns are given with the notations s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). A broad signal is indicated by the notation br. The samples were prepared by dissolving 5-7 mg of the compound in 0,5 ml deuterated solvent (CDCl_3 or DMSO). The data ejected from the spectrometer was analyzed using the software NMRNotebook (NMRTEC).

Mass spectrometry

Mass spectrometry was performed by using a ThermoScientific LCQ Deca XP (electro-spray ionization, ESI) spectrometer. The procedure was carried out by the *Centre of Excellence for Mass Spectrometry (CEMS)* at King's College London. Samples were prepared by dissolving 1 mg of the compound in 1 mL of MeOH or other suitable solvents.

Melting points

Melting points were determined on an Electrothermal IA 9100 Melting Point Apparatus and are uncorrected.

Chromatography

Thin layer chromatography was carried out using classical aluminum plates coated with silica gel 60 F₂₅₄ from Merck. When column chromatography was applied, silica gel 40-63 μm (Zeoprep) from Zeochem was used.

Chemicals

All the reagents and solvents were purchased from Sigma-Aldrich, BDH, Alfa-Aesar and Fischer Scientific in either reagent or analytical grade and used without further purification.

5 Synthetic procedures

5.1 Synthesis of 3-benzyloxy-2-ethyl-pyran-4-one

Ethyl-maltol (115,51 g, 825,07 mmol) was placed in a one-neck round bottomed flask and dissolved in 400 mL of MeOH. A solution of NaOH (36,3 g in 100 mL of water) was added and the flask was equipped with a magnetic stirrer. Benzyl chloride (113,41 mL, 990,08 mmol) was placed in an addition funnel and added dropwise to the stirred solution. The bath temperature was brought to 100° and allowed to react under reflux overnight. After 18 hours of reflux the clear solution had turned orange. The completion of the reaction was checked by TLC (DCM/MeOH 9,5:0,5). The solvent (MeOH) was removed under reduced pressure. DCM (500 mL) was added to the residue and placed in a separating funnel. First, the solution was washed with NaOH 5% (3 x 250 mL) and saturated sodium chloride solution (1 x 250 mL) and then with water (1 x 300 mL). A change of color from brown to yellow to deep red was observed. The organic phase was dried over sodium sulfate. After having filtered off the sodium sulfate, the solvent was removed under reduced pressure. The residue was dissolved in 50 mL of diethyl ether. The flask was put in the freezer overnight, but no crystals had formed until the next day. Upon scratching the walls of the flask with a glass rod, the compound started to precipitate from the solution. The crystals were filtered off through a Büchner funnel and washed with an ice-cold mixture of hexane/diethyl ether.

Yield: 124,62 g (65,60%) nearly colourless needles

Melting point: 95° C

¹H-NMR (CDCl₃): δ 0,90-0,94 (t, -CH₂CH₃, 3 H); 2,41-2,47 (q, -CH₂CH₃, 2 H); 5,10 (s, -OCH₂-, 2 H); 6,29-6,30 (d, **5**-H, 1 H); 7,24-7,33 (m, -Phe, 5 H); 7,55-7,57 (d, **6**-H, 1 H)

5.2 Synthesis of 3-Benzoyloxy-1-carboxypentyl-2-methyl-4(1H)-pyridinone

2-Ethyl-3-benzoyloxy-4-pyranone (5 g, 21,74 mmol) and 6-aminocaproic acid (8,56 g, 65,22 mmol) were placed in a one-neck round bottomed flask and dissolved in a mixture of EtOH and water (50:50). The flask was equipped with a magnetic stirrer and 10 N NaOH was added until a pH of 13 was reached. The solution was heated under reflux at 100° overnight. TLC analysis (DCM/MeOH 9,5:0,5) showed the reaction to be completed. The solvent (EtOH) was removed under reduced pressure and the remaining solution was acidified with HCl to pH 4 to achieve precipitation. The aqueous solution was washed with DCM (3 x 100 mL) and the combined organic extracts were dried over sodium sulfate. Sodium sulfate was filtered off and the solvent (DCM) was removed under reduced pressure. A viscous orange/brown liquid was obtained. The residue was dissolved in a small amount of EtOH and then diethyl ether was added until the solution went cloudy. The mixture was allowed to stand in the fridge overnight. The precipitate was collected on a Büchner funnel and washed with several small portions of diethyl ether. Yellow/brown crystals were obtained.

Yield: 5,16 g (69,14%) yellow/brown crystals

¹H-NMR (CDCl₃): δ 1,04-1,07 (t, -CH₂CH₃, 3 H); 1,36-1,42 (m, -CH₂-CH₂-CO-, 2 H); 1,65-1,73 (m, N-CH₂-CH₂-CH₂-, 4 H); 2,36-2,40 (t, N-CH₂-, 2 H); 2,58-2,63 (q, -CH₂CH₃, 2 H); 3,84-3,87 (t, -CH₂-CO-, 2 H); 5,26 (s, -O-CH₂-, 2 H); 6,68-6,70 (d, **5**-H, 1 H); 7,29-7,39 (m, -Phe, 5 H); 7,41-7,43 (d, **6**-H, 1 H), 9,96 (br s, -OH, 1 H)

MS (ESI) m/z: 344,13 [M+H]⁺

5.3 Synthesis of 3-Benzoyloxy-1-carboxyheptyl-2-methyl-4(1H)-pyridinone

2-Ethyl-3-benzoyloxy-4-pyranone (1,15 g, 5 mmol) and 8-aminooctanoic acid (1,59 g, 10 mmol) were placed in a one-neck round bottomed flask and dissolved in a mixture of EtOH and water (50:50, 8 mL). The flask was equipped with a magnetic stirrer and 1 M NaOH (0,4 g, 10 mmol) was added. Upon this, the content of the flask turned bright yellow, but not all the solid parts of the starting material dissolved at once. The solution was heated under reflux at 100° for 48 hours. TLC analysis (DCM/MeOH 9:1) showed the reaction to be completed as all the starting material had disappeared. The reaction mixture was cooled to room temperature and 6 M HCl was added until a pH value of 8 was reached. EtOH was removed under reduced pressure and the remaining aqueous solution was washed with DCM (3 x 25 mL). The aqueous phase was again acidified with 10% citric acid to achieve the precipitation of 3-benzoyloxy-1-carboxyheptyl-2-methyl-4(1H)-pyridinone. The precipitation started at a pH value of around 6,5 and lasted until pH 3 was reached. The resulting solid was filtered off and dissolved in DCM (100 mL). The organic phase was washed with saturated sodium chloride (brine) and dried over anhydrous sodium sulfate. Upon removal of the solvent, pale white crystals were obtained.

Yield: 1,38 g (75,34 %) pale, white crystals

¹H-NMR (CDCl₃): δ 0,95-0,98 (t, -CH₂CH₃, 3 H); 1,17-1,27 (m, -CH₂-CH₂-CH₂-CH₂-CO, 6 H); 1,54-1,60 (m, N-CH₂-CH₂-CH₂-, 4 H); 2,25-2,29 (t, -N-CH₂-, 2 H); 2,48-2,54 (q, -CH₂CH₃, 2 H); 3,58-3,72 (t, -CH₂-CO-, 2 H); 5,19 (s, -O-CH₂-, 2 H); 6,56-6,58 (d, **5**-H, 1 H); 7,15-7,27 (m, -Phe, 5 H); 7,33-7,36 (d, **6**-H, 1 H), 9,97 (br s, -OH, 1 H)

MS (ESI) m/z: 372,13 [M+H]⁺

5.4 Synthesis of 4-amino-4-(3-hydroxypropyl)-1,7-heptanediol (Bis-homotris)

5.4.1 Synthesis of Bis-homotris using Zn/HCl as reducing agents

Nitromethane-tris-propanol (5 g, 21,25 mmol) was dissolved in 100 mL EtOH. The reaction was started at room temperature and while 6M HCl (75 mL) was added dropwise, 16,5 g of zinc powder was added in portions. The bath temperature was slowly increased to 60°C and after the initiation of the reaction the mixture was stirred at reflux for two hours. After having checked the reaction by TLC (DCM/MeOH 9:1), the solution was filtered in order to remove the zinc powder. EtOH was evaporated under reduced pressure and the residue, a clear liquid, was dissolved in ethyl acetate (150 mL). This solution was washed with 2M sodium bicarbonate (300 mL). While adding the base, the aqueous phase turned white and creamy, at the same time bubbles of gas were welling up. Furthermore, solid potassium carbonate was added to the two phases. The white precipitate in the aqueous phase was filtered off; the ethyl acetate phase was separated from the aqueous phase and then evaporated under reduced pressure. As there was no residue found, it was obvious, that the compound had to be in the present in the aqueous phase.

The presence of amino groups in the water phase was checked with a solution of ninhydrin (1,5 g ninhydrin, 50 mL butanol, 3 mL acetic acid) on a TLC plate. The purple spots indicated the presence of amino groups. The water was evaporated under reduced pressure and dried in the vacuum drier. Then the residue was dissolved in MeOH. The solution was filtered in order to remove the excessive inorganic salt. The solvent (MeOH) was evaporated under reduced pressure, but there was still a lot of salt present.

The residue was dissolved in anhydrous isopropanol and then filtered through a sintered glass funnel to remove the excessive salt. Under reduced pressure the solvent was evaporated and a sticky yellow oil was obtained.

Yield of crude product: 2,88 g (66,07%) yellow sticky oil

5.4.2 Synthesis of Bis-homotris using Raney nickel as reducing agent

Nitromethane-tris-propanol (2,5 g, 10,6 mmol) was dissolved in 60 mL of EtOH. T-1^[41] Raney nickel (6 g) was added and hydrogenation was carried out at 40 psi. The reaction was completed within 24 hours. The Raney nickel was filtered off and the solvent was removed under reduced pressure. A yellow sticky oil was obtained.

Yield: 1,68 g (0,76,74%) slightly yellow sticky oil

¹H-NMR (DMSO): δ 1,21-1,25 (m, -CH₂-CH₂-CH₂-OH, 6 H); 1,33-1,41 (m, -CH₂-CH₂-CH₂-OH, 6 H); 3,30-3,36 (m, -CH₂-CH₂-CH₂-OH, 6 H)

MS (ESI) m/z: 206,07 [M+H]⁺

5.5 Synthesis of ((2,5-dioxopyrrolidin-1-yl) 3-(tert-butoxycarbonylamino)propanoate

Boc- β -Alanine (1 g, 5,28 mmol) and N-hydroxysuccinimide (0,61 g, 5,28 mmol) were dissolved in ethyl acetate (25 mL, dried over sodium sulfate). DCC (1,1 g, 5,28 mmol) in ethyl acetate (4 mL) was added to the stirred solution. The mixture was stirred at room temperature overnight and then stored in the fridge for a couple of hours to precipitate as much DCU as possible. The heavy white precipitate was filtered off and the filter cake was washed with cold ethyl acetate. The combined filtrates were concentrated and crystals formed at room temperature. Propan-2-ol (approximately 3 mL) was added and more crystals formed. The precipitate was allowed to stand in the fridge overnight and then filtered off and washed with cold propan-2-ol. White crystals were obtained.

Yield: 1,2 g (80%) white crystals

Melting point: 95° C

$^1\text{H-NMR}$ (CDCl_3): δ 1,38 (s, $-\text{CH}_3$, 9 H); 2,76-2,78 (m, $-\text{CH}_2\text{-COO-N}<$ and $-\text{CH}_2\text{-succinimide}$, 6 H); 3,43-3,48 (q, t-Bu-OOC-NH- CH_2 -, 2 H); 5,04 (s, $-\text{NH-}$, 1 H)

IR (KBr): 3362, 2978, 2934, 1824, 1779, 1688, 1523, 1435, 1366, 1289, 1198, 1096 cm^{-1}

5.6 Synthesis of ditert-butyl 4-(3-tert-butoxy-3-oxo-propyl)-4-[3-(9H-fluoren-9-ylmethoxycarbonylamino)propanoylamino]heptanedioate

Ditert-butyl 4-amino-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate (2,45 g, 6 mmol), Fmoc- β -alanine (1,63 g, 5 mmol), DCC (1,24 g, 6 mmol) and HOBt (0,81 g, 6 mmol) were dissolved in dimethylformamide (DMF) (25 mL) and placed in a one-neck round-bottomed flask. The solution was stirred at room temperature overnight. The solution turned slightly pink and contained white, solid DCU. The reaction was judged complete by TLC (DCM/MeOH 9:1). The white precipitate was filtered through a Büchner funnel and then the solvent (DMF) was removed under reduced pressure. Orange colored oil was obtained. The residue was dissolved in ethyl acetate and was then passed through a silica gel column in order to purify the compound. The column was eluted with ethyl acetate/hexane (1:3, increased to 1:1). Having removed the solvent of the collected fractions, white crystals were obtained.

Yield: 3,48 g (98,30%) white crystals

$^1\text{H-NMR}$ (CDCl_3): δ 1,36 (s, $-\text{CH}_3$, 18 H); 1,37 (s, $-\text{CH}_3$, 9 H); 1,89-1,93 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}-$, 6 H); 2,12-2,16 (m, $-\text{CH}_2-\text{CH}_2-\text{COO}-$, 6 H); 2,29-2,33 (m, $-\text{CH}_2-\text{COO}-\text{NH}-\text{C}$, 2 H); 3,41 (t, $-\text{Fmoc-NH}-\text{CH}_2-$, 2 H); 4,14-4,15 (m, **9**-H Fmoc, 1 H); 4,29 (d, $-\text{CH}_2-\text{OOC}-\text{NH}-$, 2 H); 7,24 (m, **2**-H and **7**-H Fmoc, 2 H); 7,31-7,34 (m, **3**-H and **6**-H Fmoc, 2 H); 7,52-7,54 (d, **1**-H and **8**-H Fmoc, 2 H); 7,68-7,70 (d, **4**-H and **5**-H Fmoc, 2 H)

IR (KBr): 3371, 3313, 2976, 1722, 1688, 1662, 1535, 1450, 1245, 1153, 1105 cm^{-1}

MS (ESI) m/z : 731, 20 $[\text{M}+\text{Na}]^+$

5.7 Synthesis of ditert-butyl 4-[3-(tert-butoxycarbonylamino)propanoylamino]-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate

Ditert-butyl 4-amino-4-(3-tert-butoxy-3-oxo-propyl)heptanedioate (1, 25 g, 3 mmol), Boc- β -alanine (0,57 g, 3 mmol), DCC (0,62 g, 3 mmol) and HOBt (0,41 g, 3mmol) were dissolved in DMF (20 mL) and placed in a one-neck round-bottomed flask. The solution was stirred at room temperature for 48 hours. The solution turned pink and contained white, solid precipitate (DCU). The reaction was judged complete by TLC (DCM/MeOH 9:1, iodine and ninhydrin dyes were used). The white precipitate was filtered through a Büchner funnel and then the solvent (DMF) was removed under reduced pressure. Red colored oil was obtained. The residue was dissolved in ethyl acetate and was then passed through a silica gel column in order to purify the compound. The column was eluted with ethyl acetate/hexane (1:1). Having removed the solvent of the collected fractions, white crystals were obtained.

Yield: 1,35 g (76,76%) white crystals

$^1\text{H-NMR}$ (CDCl_3): δ 1,37 (s, $-\text{CH}_3$, 36 H); 1,88-1,92 (t, $-\text{CH}_2\text{-CH}_2\text{-COO-tBu}$, 6 H); 2,12-2,16 (t, $-\text{CH}_2\text{-CH}_2\text{-COO-tBu}$, 6 H); 2,25-2,27 (t, $-\text{CH}_2\text{-CO-NH-}$, 2 H); 3,27-3,32 (q, $-\text{OOC-NH-CH}_2\text{-}$, 2 H);

IR (KBr): 3389, 3340, 2985, 1730, 1695, 1649, 1544, 1524, 1367, 1253 cm^{-1}

MS (ESI) m/z : 609,20 $[\text{M}+\text{Na}]^+$

5.8 Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-nitro-heptyl] acetate

Nitromethane-tris-propanol (2g, 8,5 mmol) was placed in a one-neck round bottomed flask and suspended in acetic anhydride (6,8 mL, 72,3 mmol). The mixture (nitromethane-tris-propanol did not dissolve completely at room temperature) was stirred and pyridine (0,07 mL, 0,85 mmol) was added. The solution was heated to 70 °C for 12 hours (during this time the nitromethane-tris-propanol dissolved completely) and a clear and yellow solution was obtained. The reaction was monitored by TLC (DCM/MeOH, 9:1) and judged to be completed when all the starting material had gone. After the heating, the flask was allowed to cool down to room temperature and poured into water (40 mL). The solution was stirred for three hours in order to quench the reaction. Sodium hydrogen carbonate was added as a solid to the stirred solution until a neutral pH value was reached. The oily yellow phase was extracted from the aqueous phase by extraction with ether (3x25 mL). The combined organic extract was washed with a solution of saturated sodium chloride and then dried over anhydrous sodium sulfate. The solvent and the remaining pyridine were removed under reduced pressure to afford the triacetate as yellow oil.

Yield: 2,72 g (70,23%) yellow oil

¹H-NMR (CDCl₃): δ 1,46-1,53 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 1,91-1,95 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 2 (s, -CH₃, 9 H); 3,99-4,02 (t, -CH₂-CH₂-CH₂-OAc, 6 H)

MS (ESI) m/z: 384,00 [M+Na]⁺

5.9 Synthesis of [7-acetoxy-4-(3-acetoxypentyl)-4-amino-heptyl] acetate

Commercially available raney nickel slurry in water (5 g) was washed with EtOH and then added to a solution of [7-acetoxy-4-(3-acetoxypentyl)-4-nitro-heptyl] acetate (2,72 g, 7,53 mmol) in EtOH (55 mL) and hydrogenated at 40 psi. After the hydrogen uptake had ceased (approximately 24 hours) the Raney nickel was carefully filtered off and the solvent was removed in vacuo. A slightly yellow oil was obtained (2,3 g, 92%) which was then purified by silica gel column chromatography using DCM/MeOH (8,5:2,5) as eluent. The residue was a slightly yellow oil.

Yield: 1,7 g (70,88%) slightly yellow oil

¹H-NMR (CDCl₃): δ 1,29-1,34 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 1,51-1,57 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 1,98 (s, -CH₃, 9 H); 3,98-4,01 (t, -CH₂-CH₂-CH₂-OAc, 6 H)

IR: 3368, 2955, 1736, 1455, 1365, 1242, 1036 cm⁻¹

MS (ESI) m/z: 332,00 [M+H]⁺

5.10 Synthesis of [7-acetoxy-4-(3-acetoxypropyl)-4-[3-(tert-butoxycarbonylamino)propanoylamino]heptyl] acetate

Boc- β -Alanine (0,99 g, 5,28 mmol) and [7-acetoxy-4-(3-acetoxypropyl)-4-amino-heptyl] acetate (1,75 g, 5,28 mmol) were placed in a one-neck round bottomed flask and dissolved in anhydrous DMF (20 mL). DCC (1,09 g, 5,28 mmol) and HOBt (0,71 g, 5,28 mmol) were added and the solution was stirred at 25 °C overnight. Precipitate formed after approximately 15 min and the solution turned slightly yellow. The reaction was monitored by TLC (ethyl acetate/hexane 1:1) and judged to be complete after 24 hours. The dicyclohexylurea was filtered through Celite and the remaining solution was concentrated in vacuo to give a sticky yellow oil. This residue was dissolved in DCM (60 mL) and sequentially washed with cold aqueous 10% HCl (30 mL), water (30 mL), aqueous 10% NaHCO₃ (30 mL) and a solution of saturated NaCl (30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. A thick, viscous residue was obtained. For further purification, the residue was passed through a silica gel column, eluting with ethyl acetate/hexane (1:1). The polarity of the solvent was increased by using only ethyl acetate. White crystals were obtained.

Yield: 2,2 g (83,02%) white crystals

Melting point: 90° C

¹H-NMR (CDCl₃): δ 1,36 (s, (CH₃)₃-C-OOC-, 9 H); 1,47-1,48 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 1,65-1,66 (m, -CH₂-CH₂-CH₂-OAc, 6 H); 1,98 (s, -O-OC-CH₃, 9 H); 2,28-2,29 (t, -CH₂-CO-NH-, 2 H); 3,27-3,29 (q, -OOC-NH-CH₂-, 2 H); 3,95-3,98 (t, -CH₂-CH₂-CH₂-OAc, 6 H)

IR (KBr): 3287, 2979, 1738, 1684, 1642, 1545, 1385, 1367, 1243, 1174, 1051 cm⁻¹

MS (ESI) m/z: 525,25 [M+Na]⁺

5.11 Synthesis of tert-butyl N-[3-[[4-hydroxy-1,1-bis(3-hydroxypropyl)butyl]amino]-3-oxo-propyl]carbamate

A mixture of the triacetate (2,17 g, 4,3 mmol) and anhydrous potassium carbonate (0,19 g, 1,4 mmol) was placed in a one-neck round bottomed flask and dissolved in absolute EtOH (27 mL). The solution was stirred at 30 °C for 18 hours and monitored by TLC, using ethyl acetate as eluent. The spots were revealed by both iodine and ninhydrin dye. The potassium carbonate dissolved only very slowly and even after 18 hours there was still some solid left. When all the starting material had gone, the fine precipitate that had formed was filtered through Celite and the solvent was reduced in vacuo. The residue was viscous, white oil that solidified when it was stored in a high vacuum desiccator.

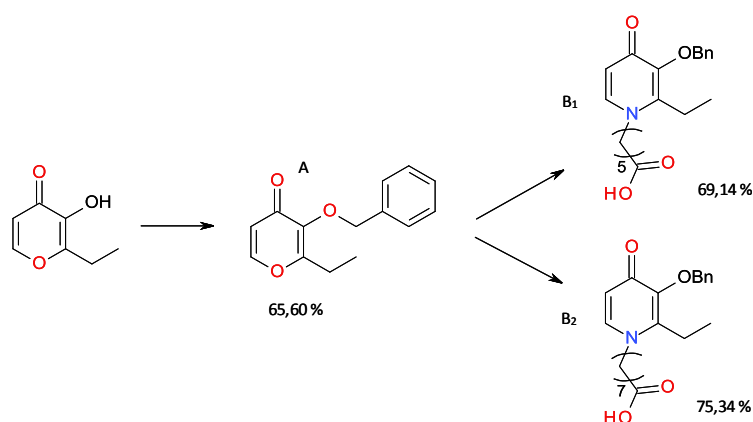
Yield: 1,59 g (98,15%) white solid

¹H-NMR (CDCl₃): δ 1,25-1,33 (m, -CH₂-CH₂-CH₂-OH, 6 H); 1,37 (s, (CH₃)₃-C-OOC-, 9 H); 1,53-1,58 (m, -CH₂-CH₂-CH₂-OH, 6 H); 2,18-2,21 (t, -CH₂-CO-NH-, 2 H); 3,04-3,09 (q, -OOC-NH-CH₂-, 2 H); 3,32-3,33 (t, -CH₂-CH₂-CH₂-OH, 6 H)

IR (KBr): 3500-3000, 3329, 2940, 1693, 1548, 1367, 1252, 1170, 1054 cm⁻¹

MS (ESI) m/z: 399,25 [M+Na]⁺

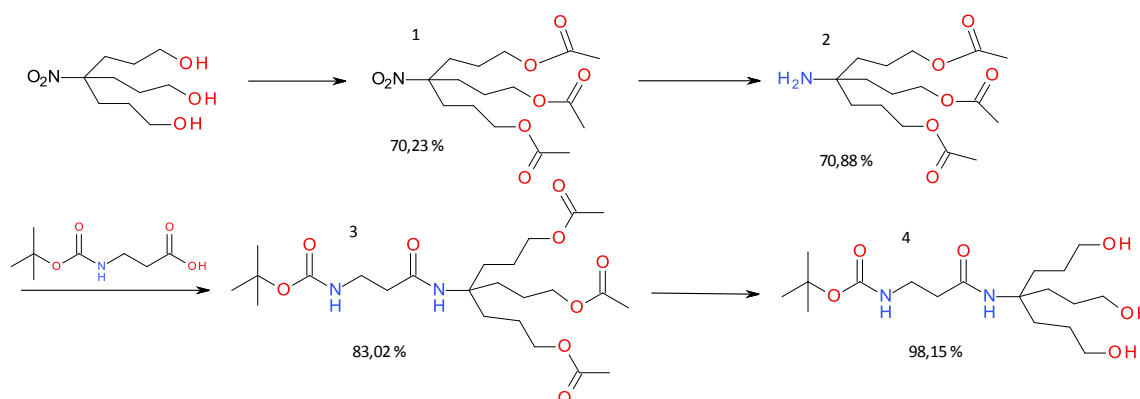
6 Short summary of the synthetic achievements and outlook on further steps



Scheme 25: Synthesis of the HPO-derivatives

Scheme 25 shows the synthetic pathway for the two benzyl-protected HPO-derivatives. Avoiding column chromatography, compound **B**₁ and **B**₂ could be synthesized by precipitation from solution in good yields.

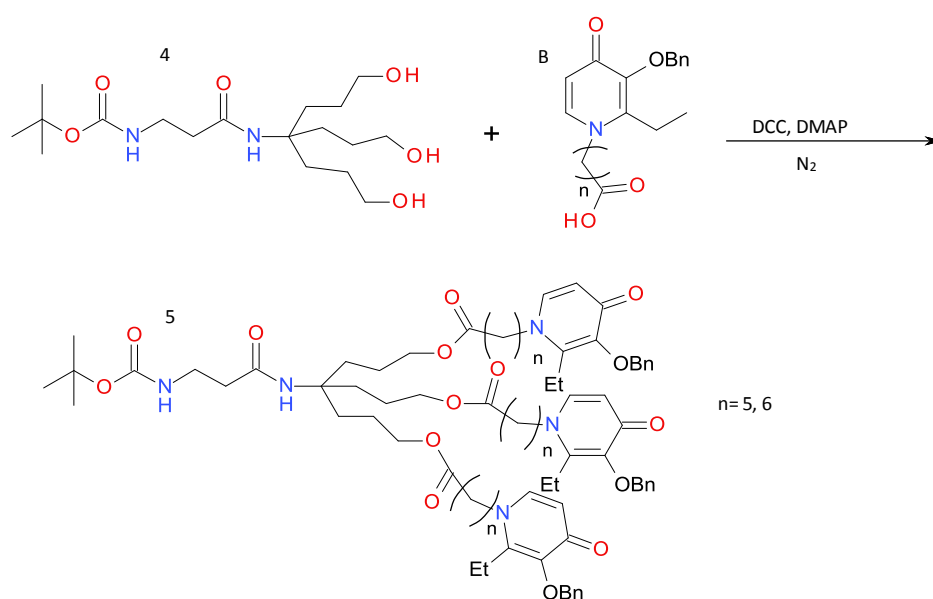
In further synthetic procedures it was initially attempted to prepare the triol building block **4** (Scheme 26) from coupling unprotected bis-homotris to Boc- β -Ala-OH or Fmoc- β -Ala-OH. When these approaches failed, the tertiary butyl-ester protected branching unit was successfully coupled with both Boc- β -Ala-OH and Fmoc- β -Ala-OH. Since the removal of the esters did not work in both cases, it was decided to introduce another protective group for the triol branching unit.



Scheme 26: Synthetic Pathway for the synthesis of the triol **4** in four steps

Scheme 26 illustrates the pathway that leads to the desired triol building block **4** in four steps. The introduction of the acetate esters (**1**) turned out to be a convenient method for the protection of the triol. Hydrogenation of the nitro group (**2**), coupling with the linking unit Boc- β -Ala-OH (**3**) and saponification of the triacetate (**4**) were easily achieved in a relatively high overall yield.

Due to the limited time that was available for my project, it was not possible to completely finish the desired dendron (compound **5**, Scheme 27). The first attempt to couple the HPO-derivative **B**₁ to the triol building block **4** was only partially successful.



Scheme 27: Synthesis of the dendron: the last step using DCC and DMAP under a nitrogen atmosphere

It is still necessary to work on the conditions of this procedure (Scheme 27) in order to achieve a more quantitative reaction. A chemist of the department of Medicinal Chemistry at KCL will carry on improving this last reaction with the aim of obtaining both of the desired dendrons in an acceptable yield and purity.

Thus, the successful development of the previously described, convenient 4-step synthetic pathway will facilitate the synthesis of similar dendrons bearing more generations or other terminal groups. All of these compounds will be tested in biological essays for their suitability in photodynamic therapy.

7

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7.5 List of Abbreviations

$^1\text{H-NMR}$	^1H - nuclear magnetic resonance
Å	angstrom
ALA / 5-ALA	5-aminolevulinic acid
aminocaproic acid	6-aminohexanoic acid
bis-homotris	4-amino-4-(3-hydroxypropyl)-1,7-heptanediol
Boc	<i>tert</i> -butyloxycarbonyl
CoA	Coenzyme A
CP94	1,2-diethyl-3-hydroxypyridin-4-one
DCC	N,N'-dicyclohexyl-carbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
EPR	enhanced permeation and retention
ESI	electrospray ionization
ethyl maltol	2-ethyl-3-hydroxy-pyran-4-one
EtOH	ethanol
Fmoc	fluorenylmethyloxycarbonyl
HCl	hydrochloric acid
HOBT	1-hydroxy-benzotriazole hydrate
HpD	Haematoporphyrin derivative
HPO	3-hydroxypyridin-4-one
IR	infrared
KBr	potassium bromide
KCL	King's College London
K_2CO_3	potassium carbonate

kg	kilogram
LiAlH ₄	lithium aluminum hydride
MeOH	methanol
Me ₄ Si	tetramethylsilane
mg	milligram
MHz	megahertz
mL	milliliter
mmol	millimole
MRI	magnetic resonance imaging
MS	mass spectrometry
MW	molecular weight
m/z	mass-to-charge ratio
NaHCO ₃	sodium hydrogen carbonate
NaOH	sodium hydroxide
nitromethane-tris-propanol	4-(3-hydroxypropyl)-4-nitro-1,7-heptanediol
PBG	porphobilinogen
PBGD	porphobilinogen desaminase
PDT	photodynamic therapy
PpIX	protoporphyrin IX
ppm	parts per million
psi	pound-force per square inch
r. a.	relative abundance
ROS	reactive oxygen species
THF	tetrahydrofuran
TLC	thin layer chromatography
Zn	zinc

7.6 Curriculum vitae

Personal details

Name: Anna Pöschl
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Academic studies

Since Oct 2006 Diploma study at the University of Vienna
Feb 2010 – Jun 2010 Erasmus student at King's College London
Department of Medicinal Chemistry
Diploma thesis project

Education

Sept 1998 – Jun 2006 Grammar school Waidhofen/Thaya
Feb 2005 – Aug 2005 Exchange semester in Brisbane, Australia
Bracken Ridge State High School
Sept 1994 – Jun 1998 Primary school Waidhofen/Thaya

Work experience

Aug 2008 Internship at the pharmacy "Apotheke zum Schwarzen Adler"
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Sept 2010 Internship at the pharmacy "Lindenapotheke" in 1170 Vienna

Language skills

German – native
English – fluent (spoken and written)
French – moderate
Spanish – basic

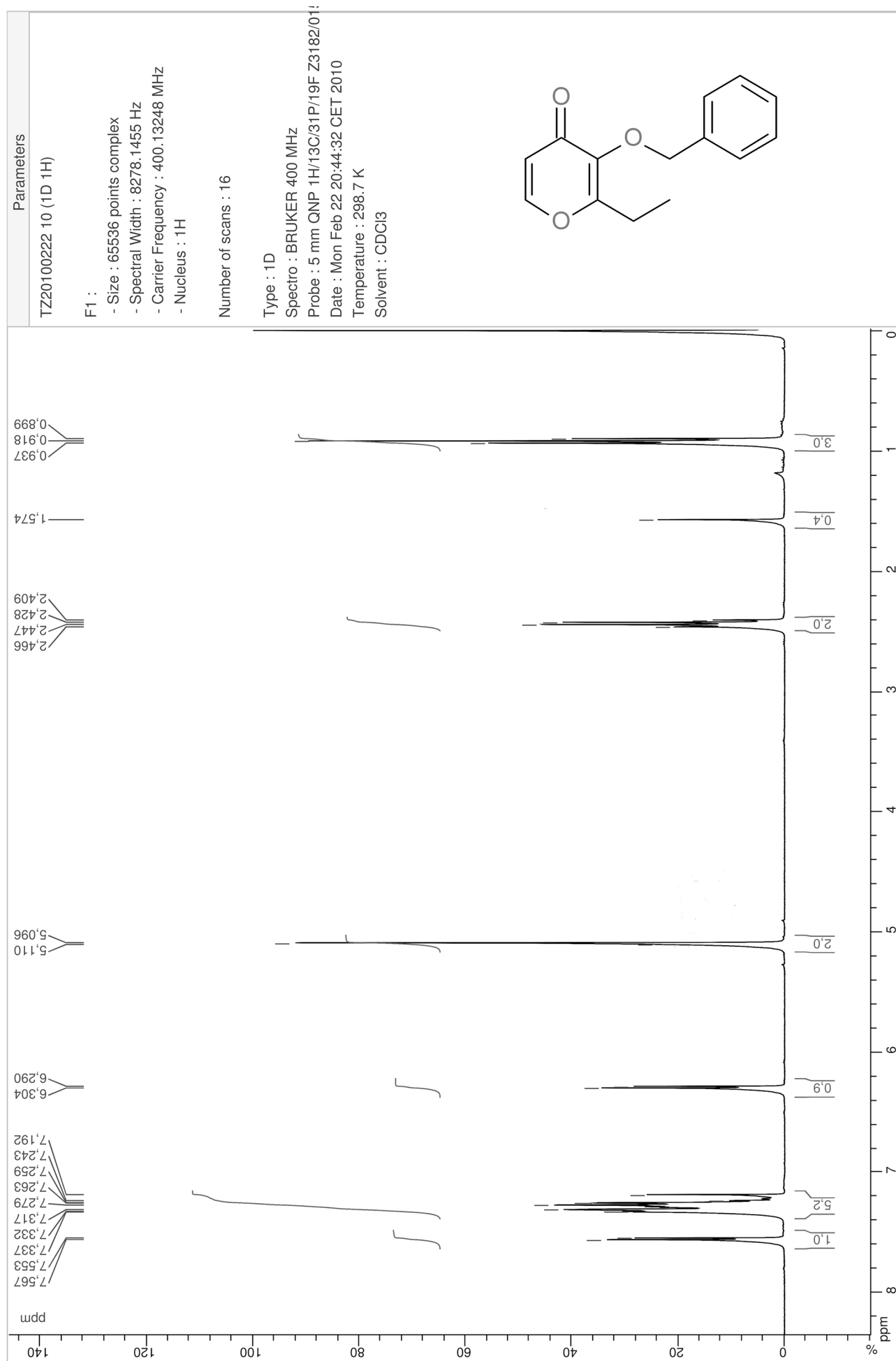
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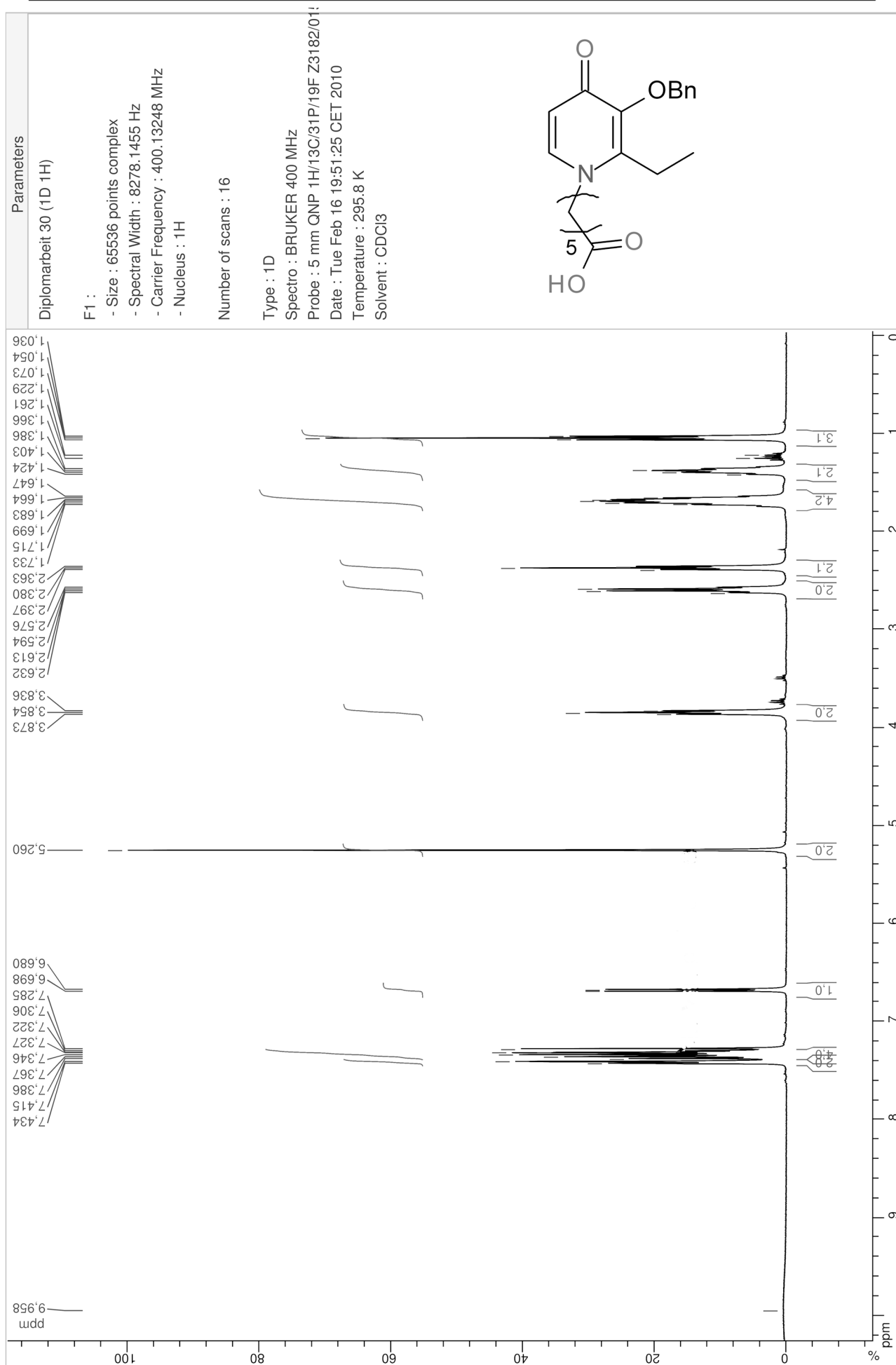
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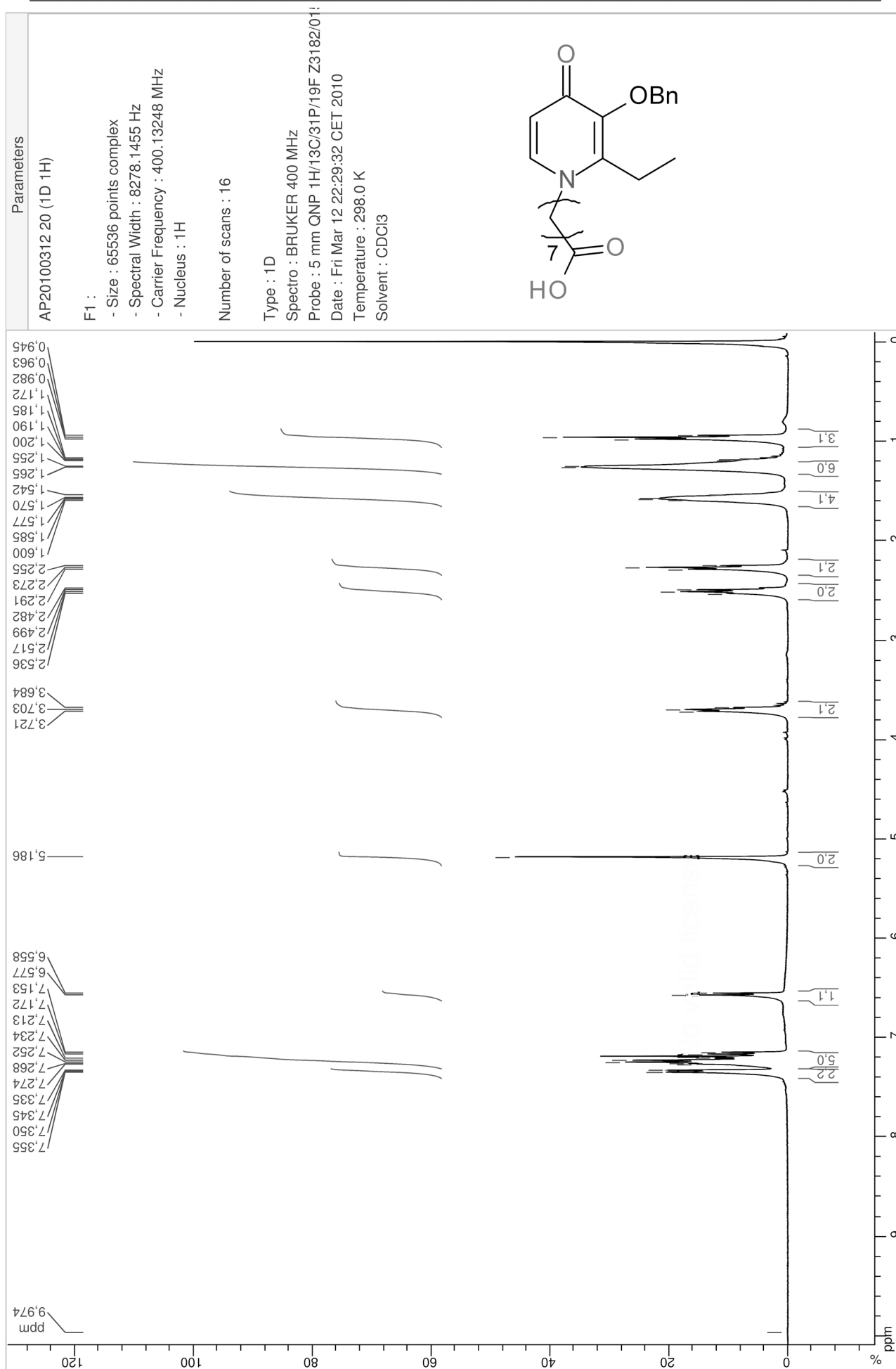
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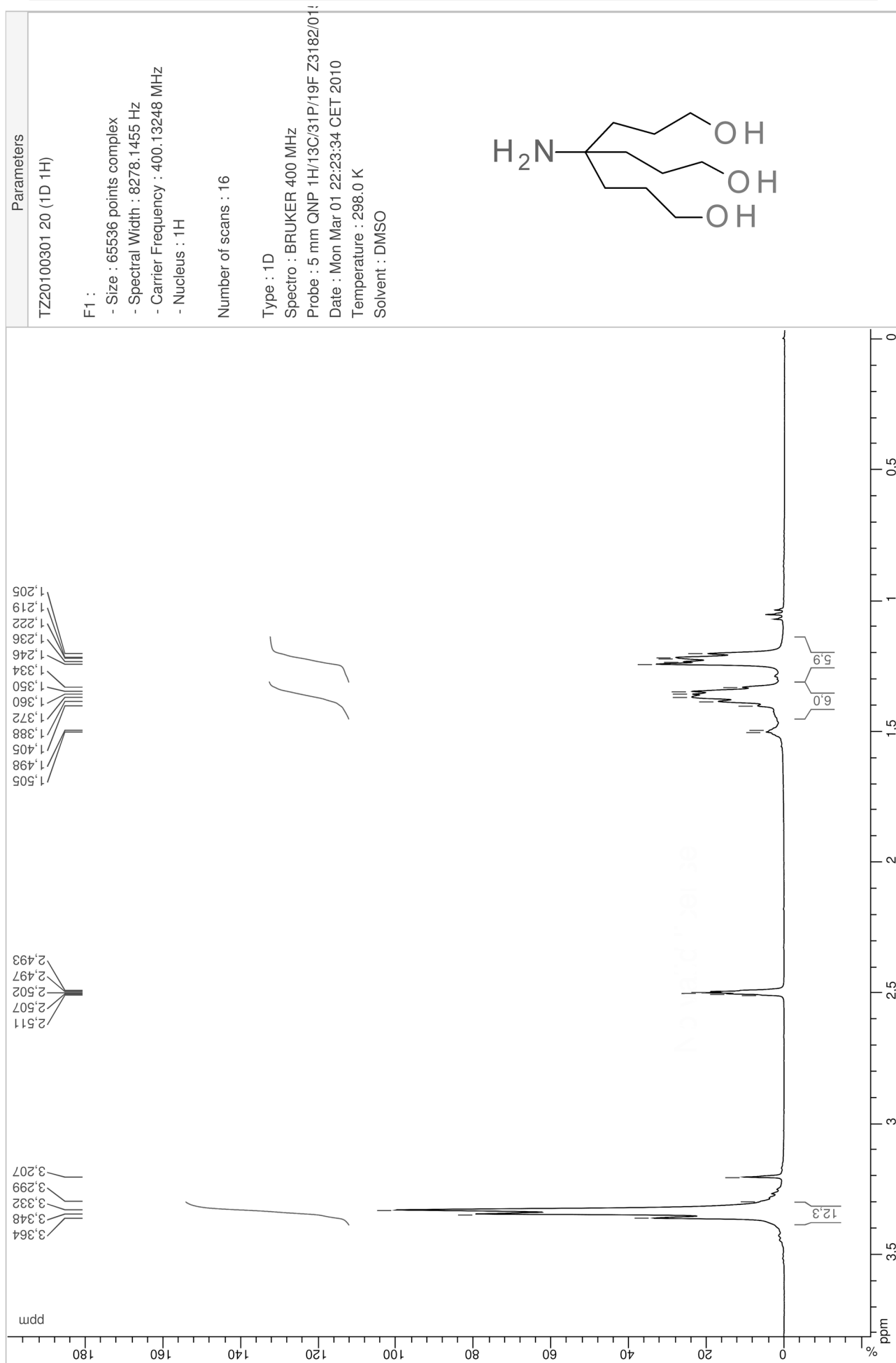


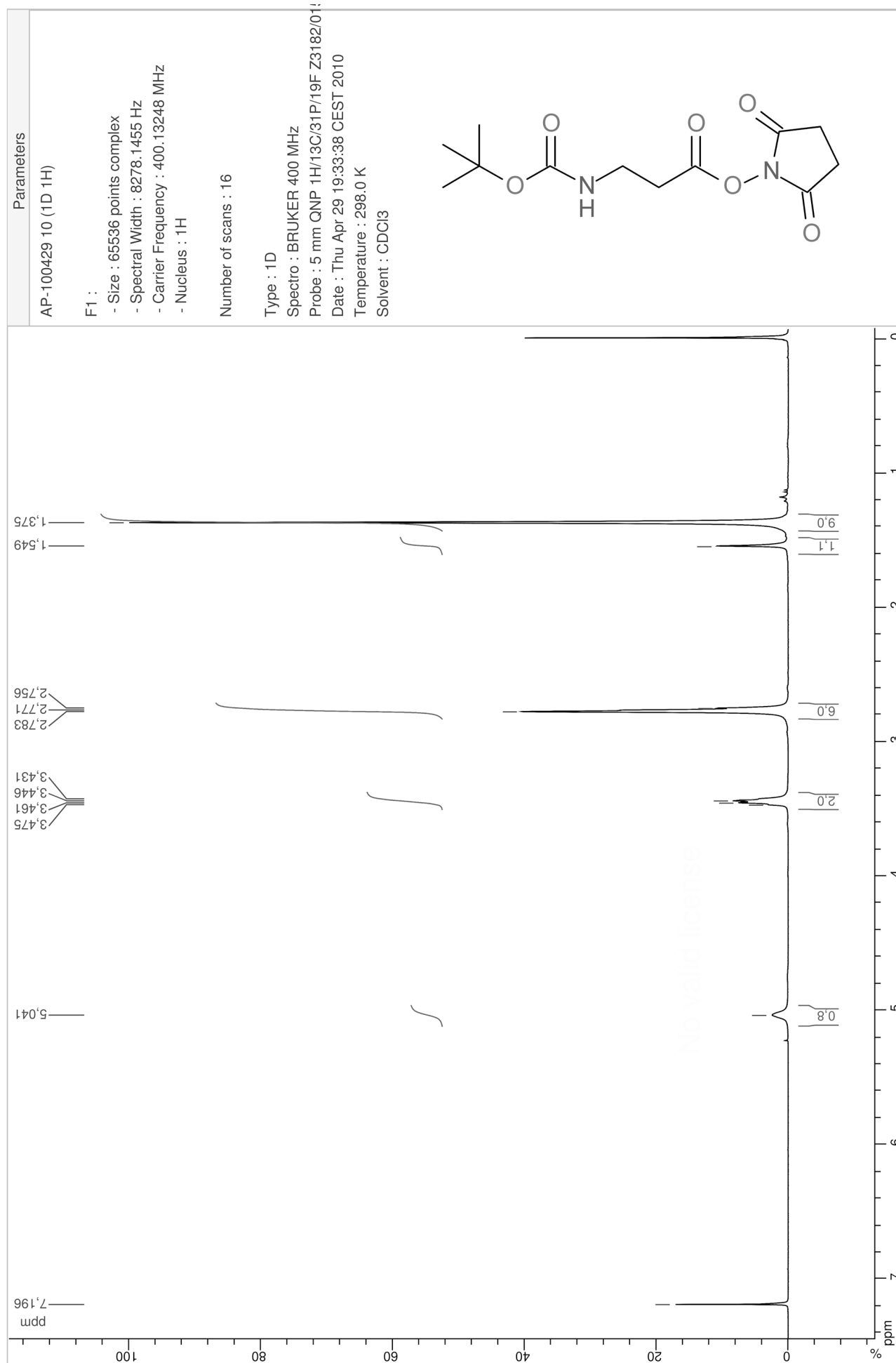
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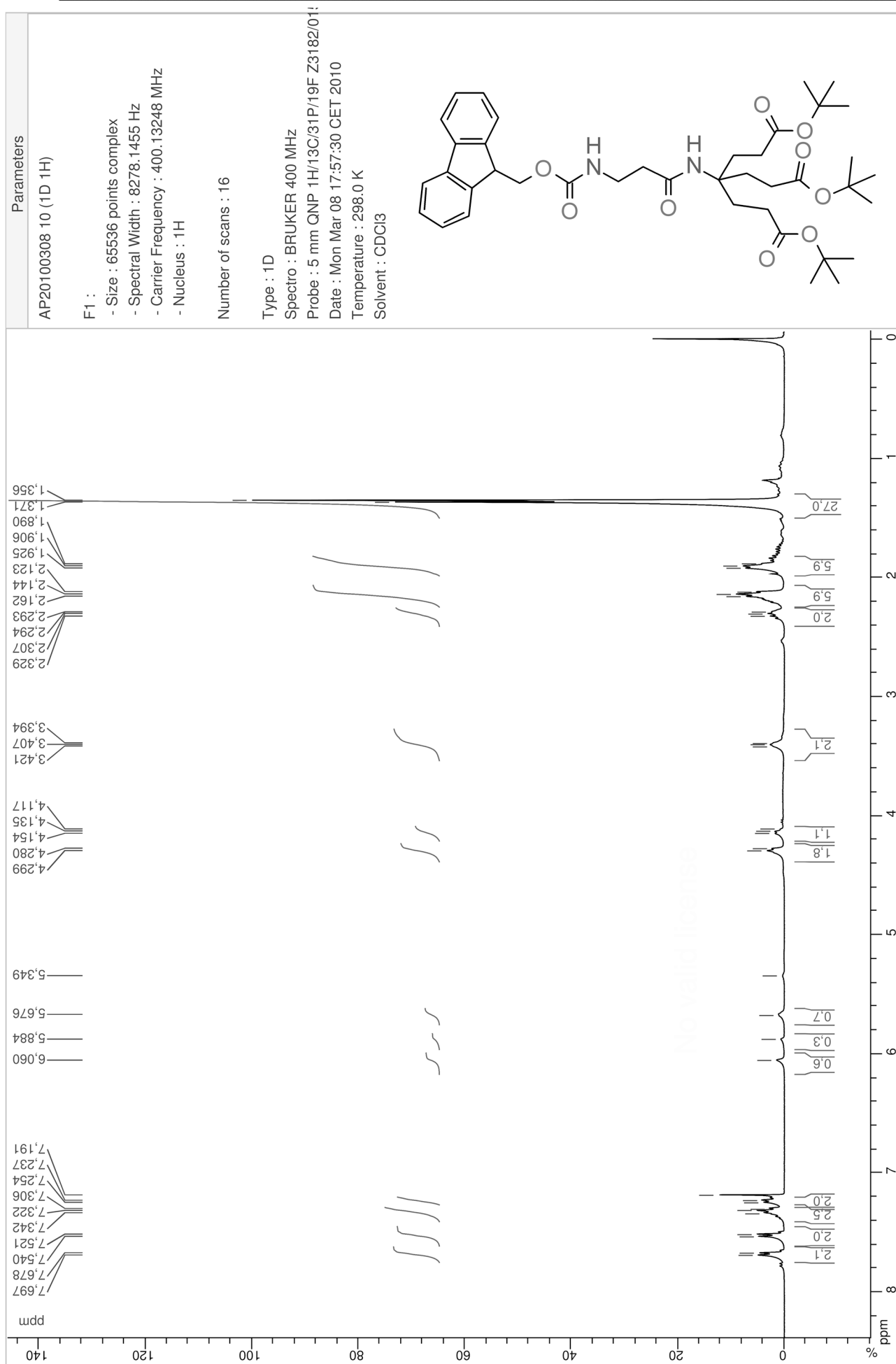


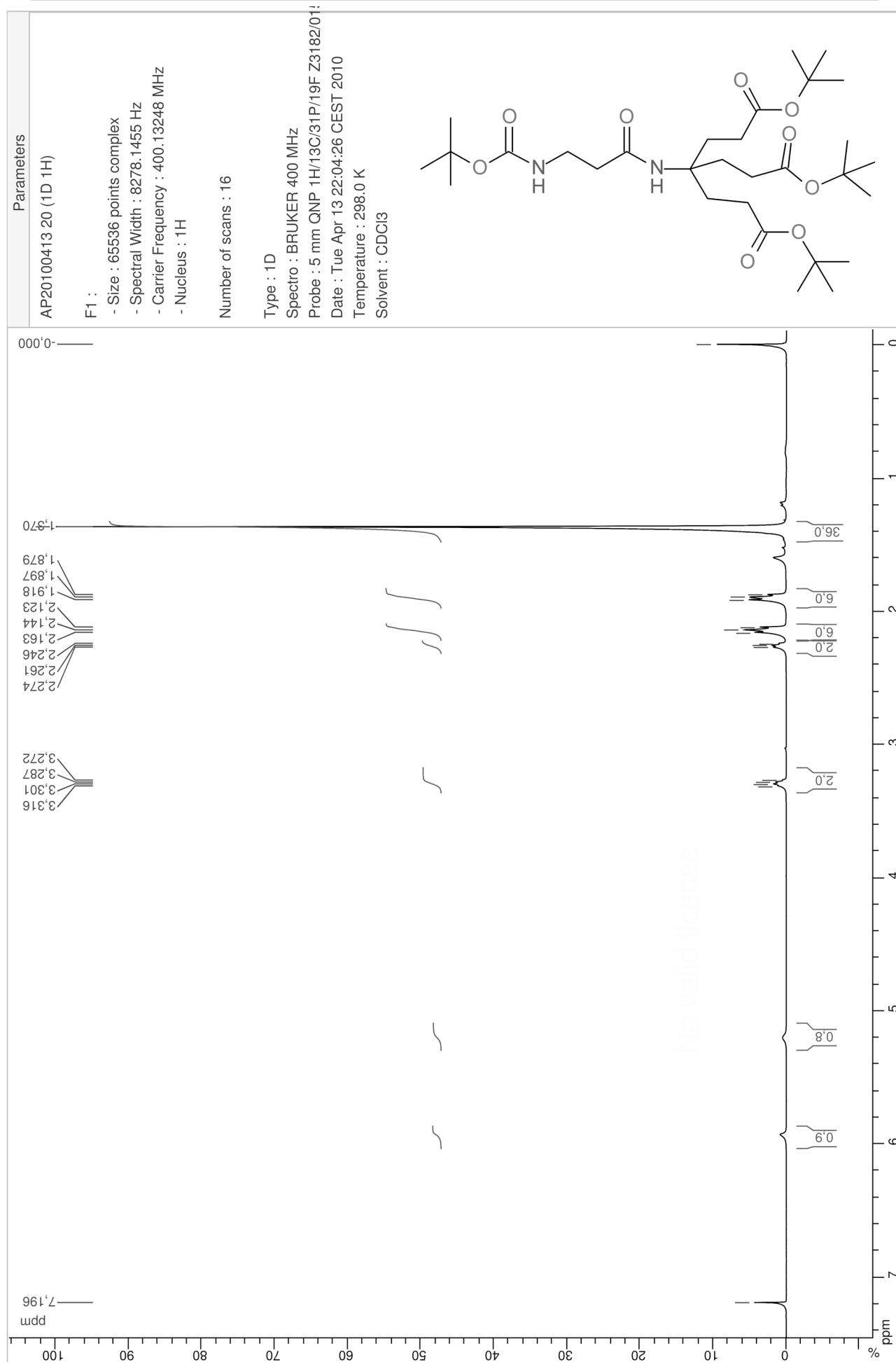


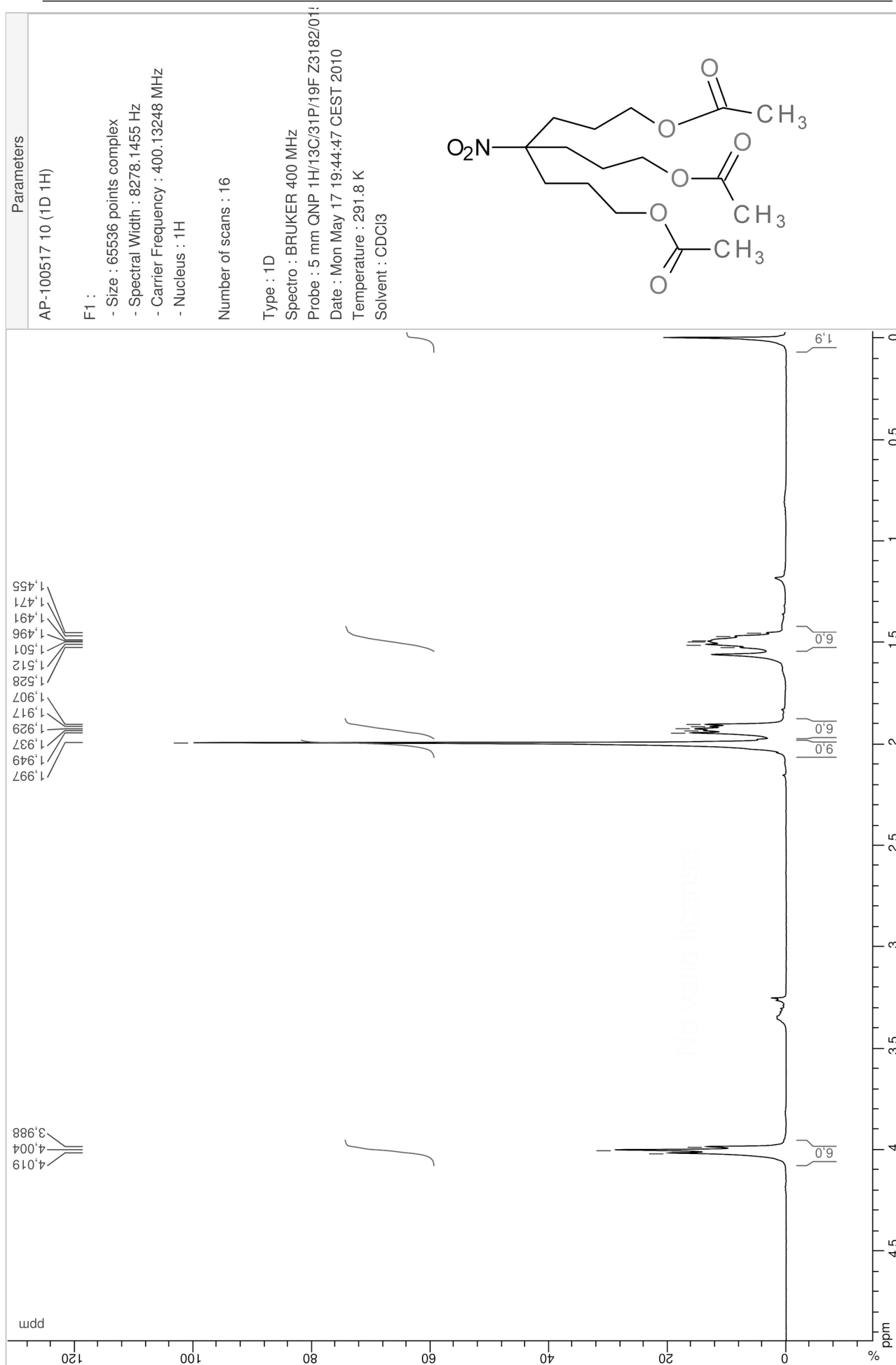


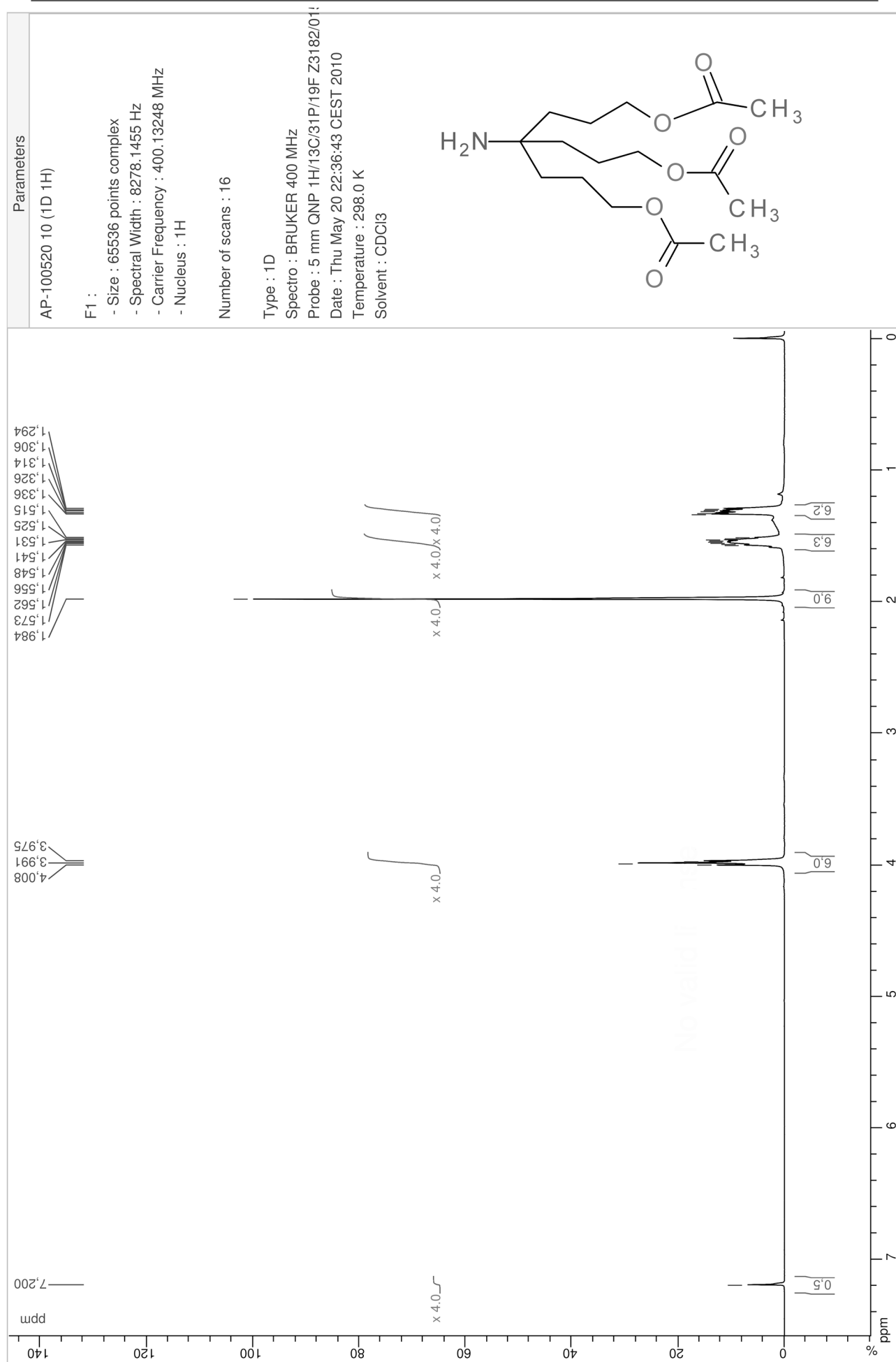


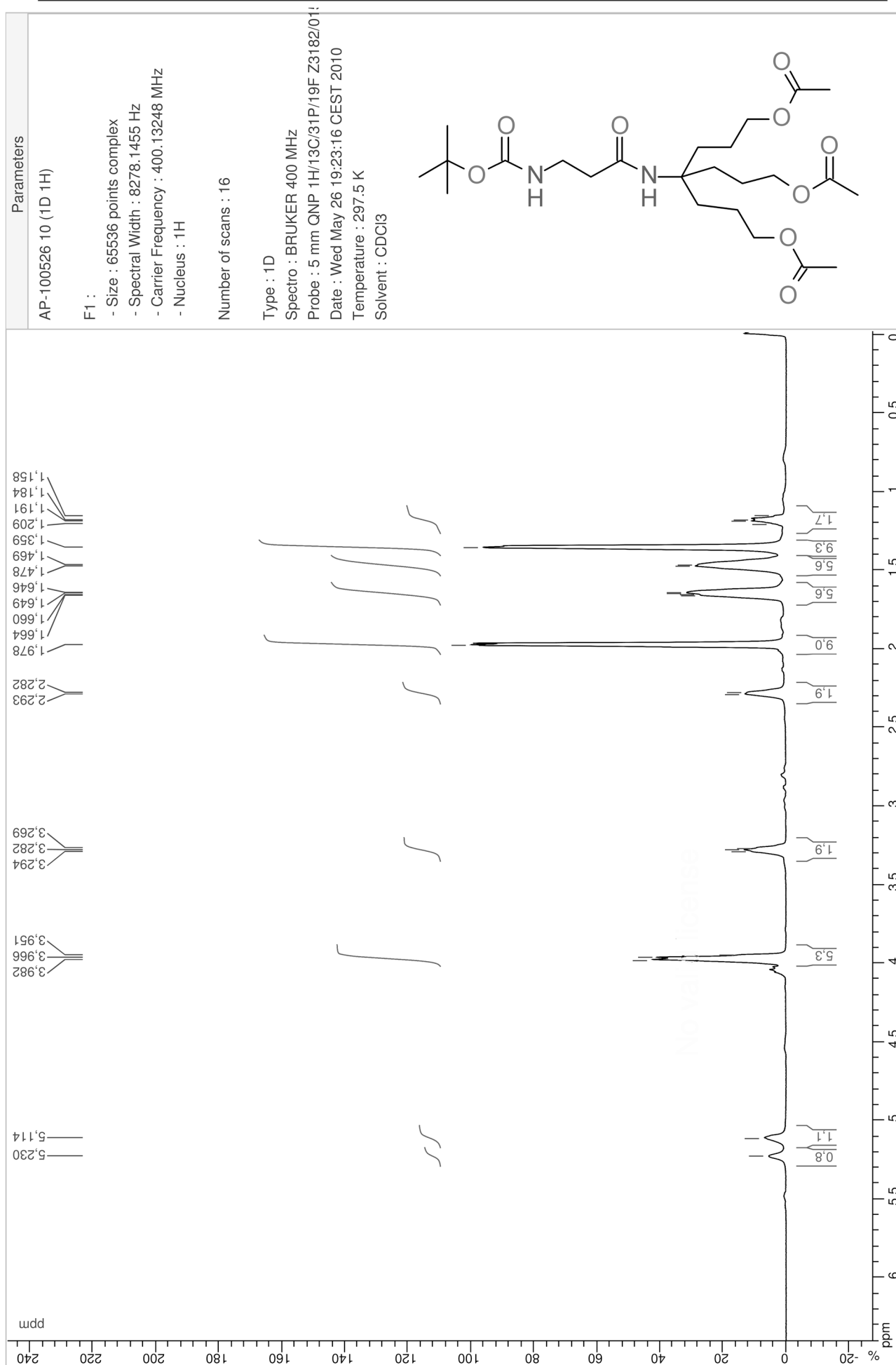


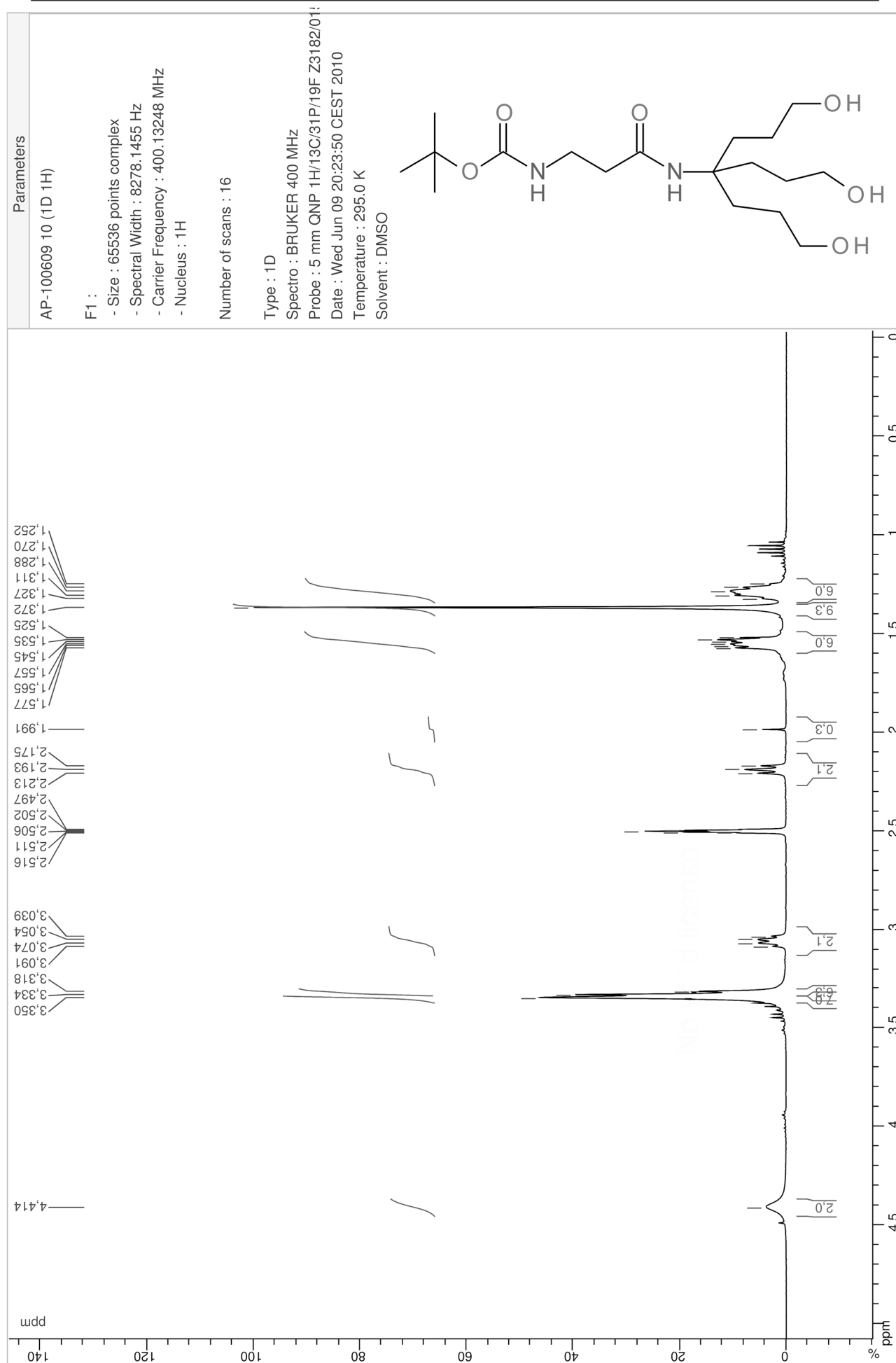


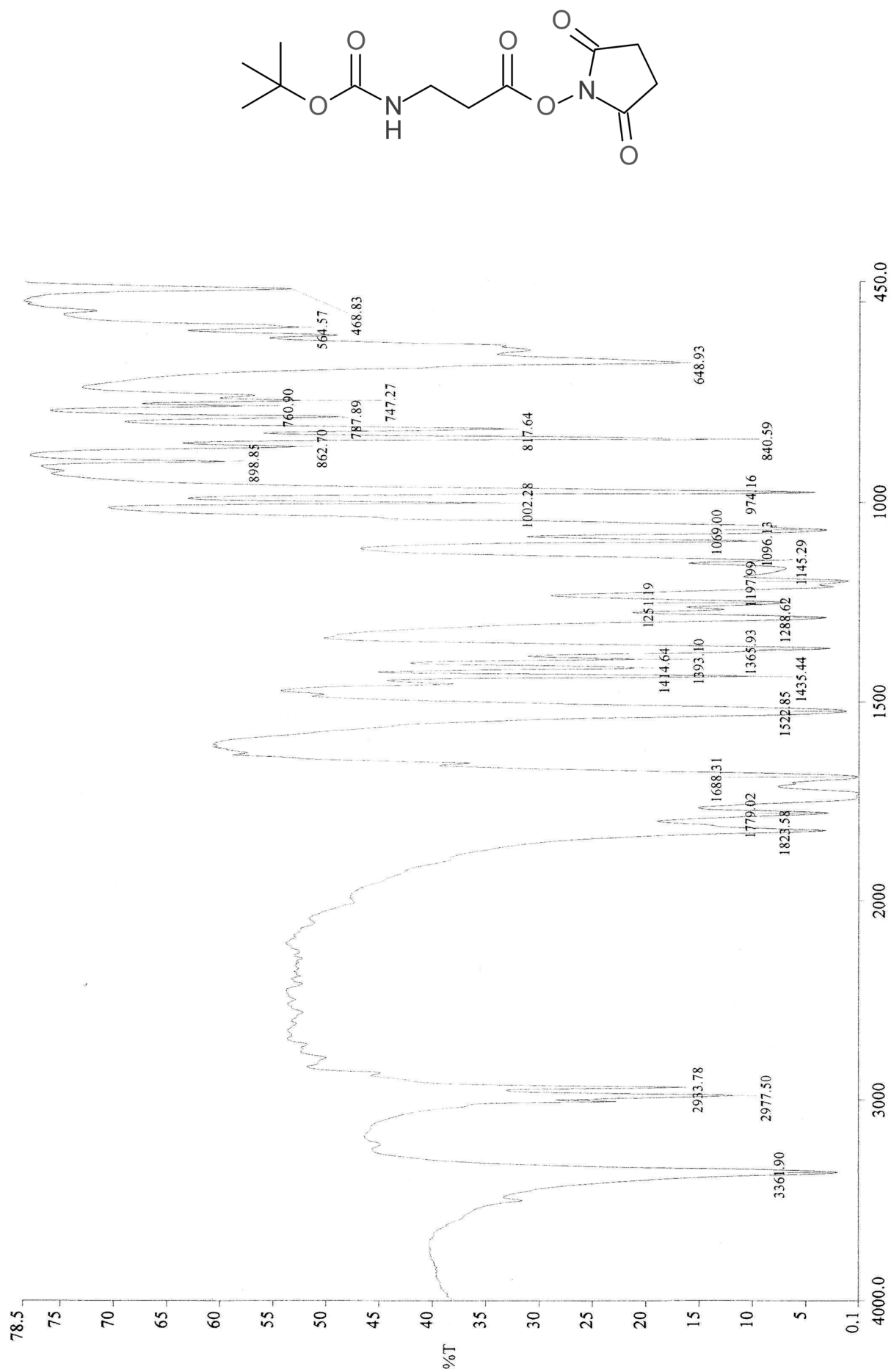


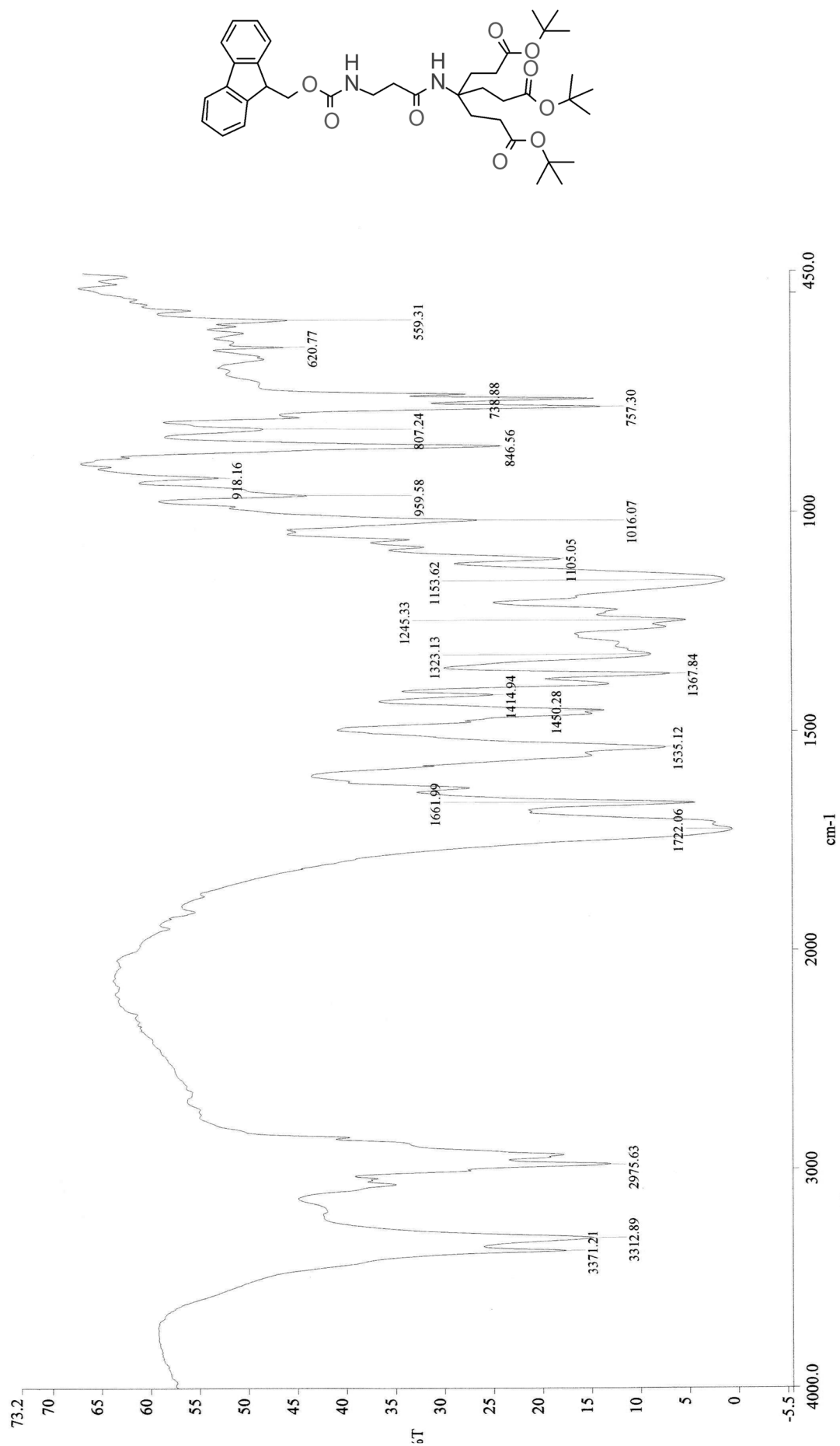


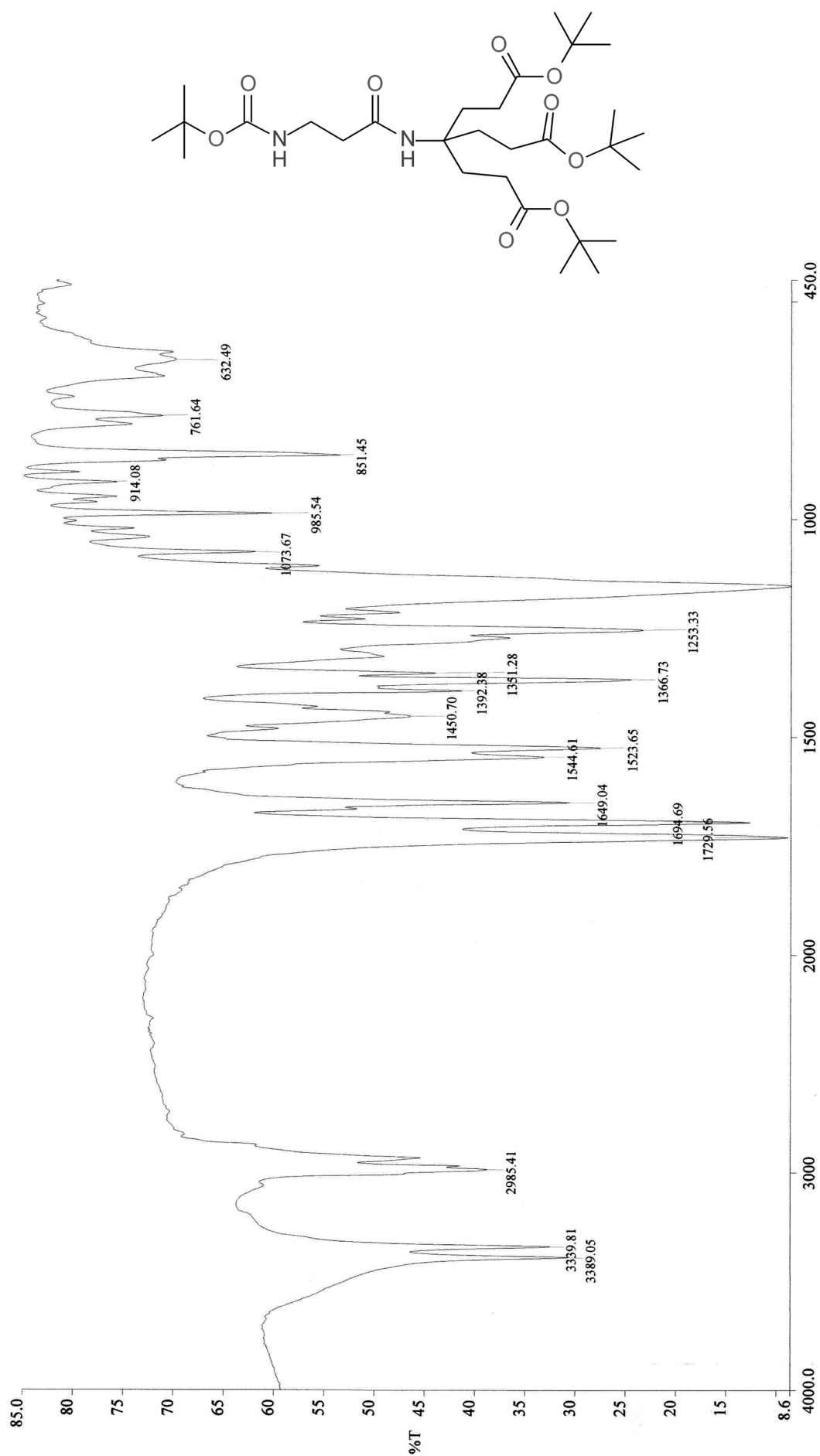


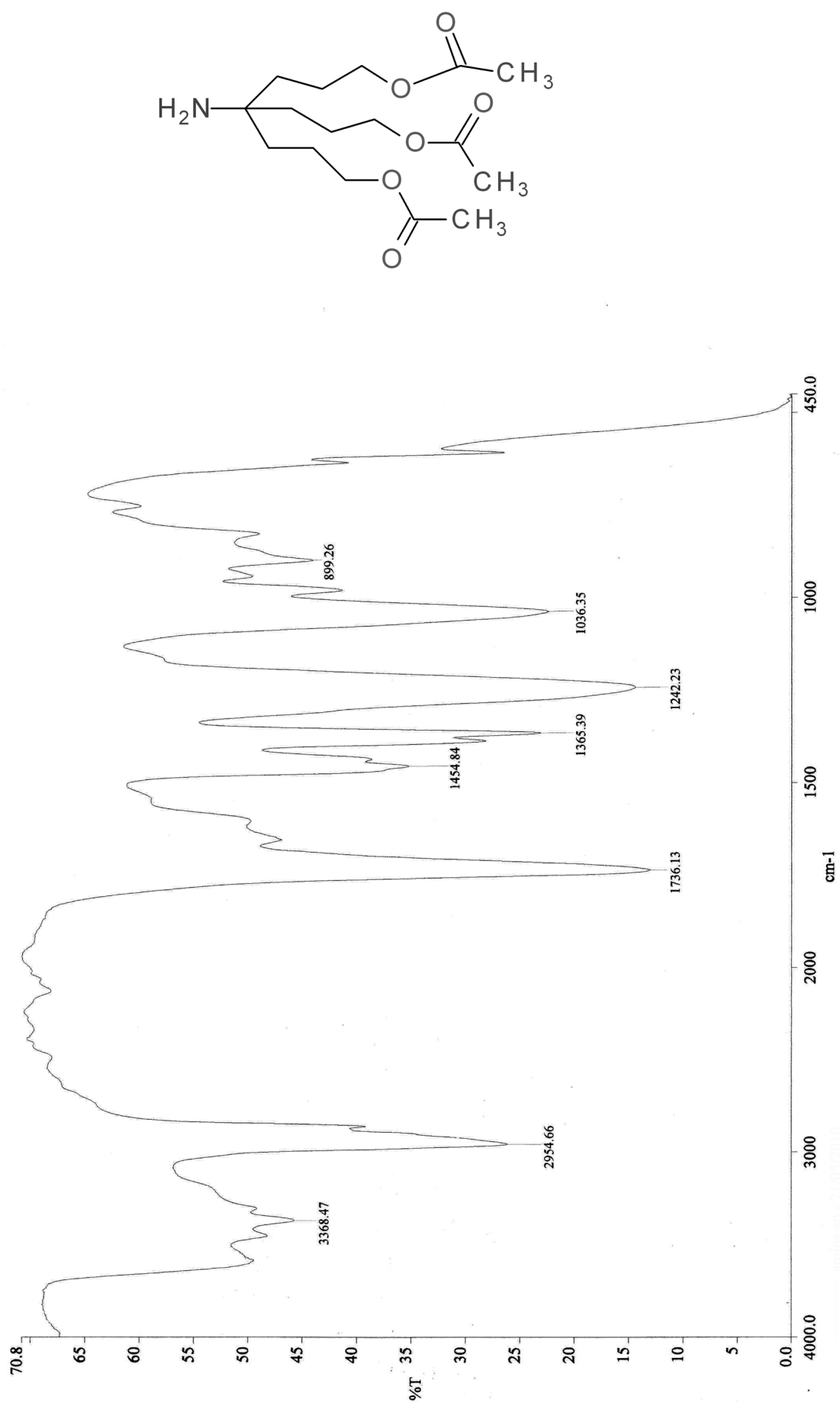


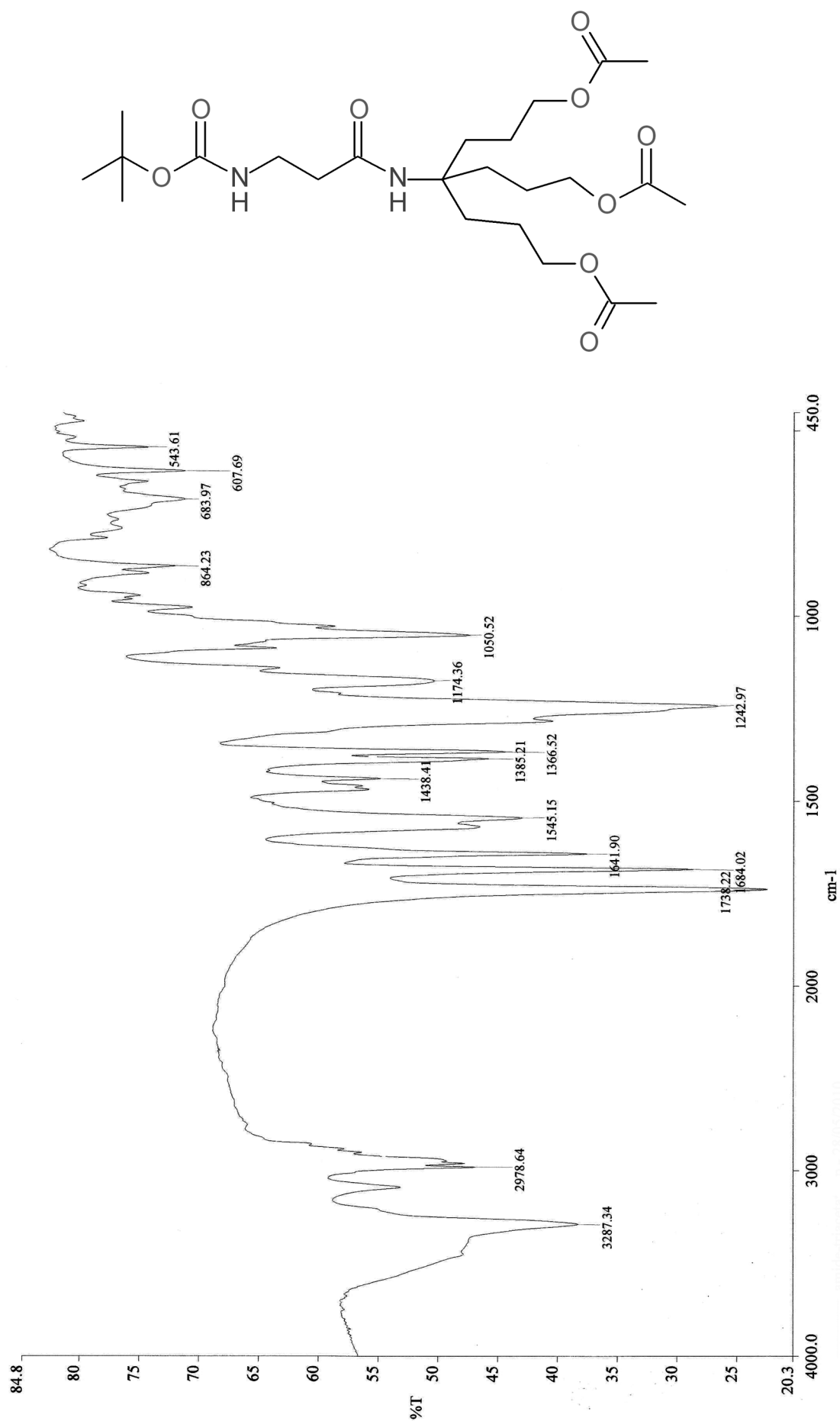


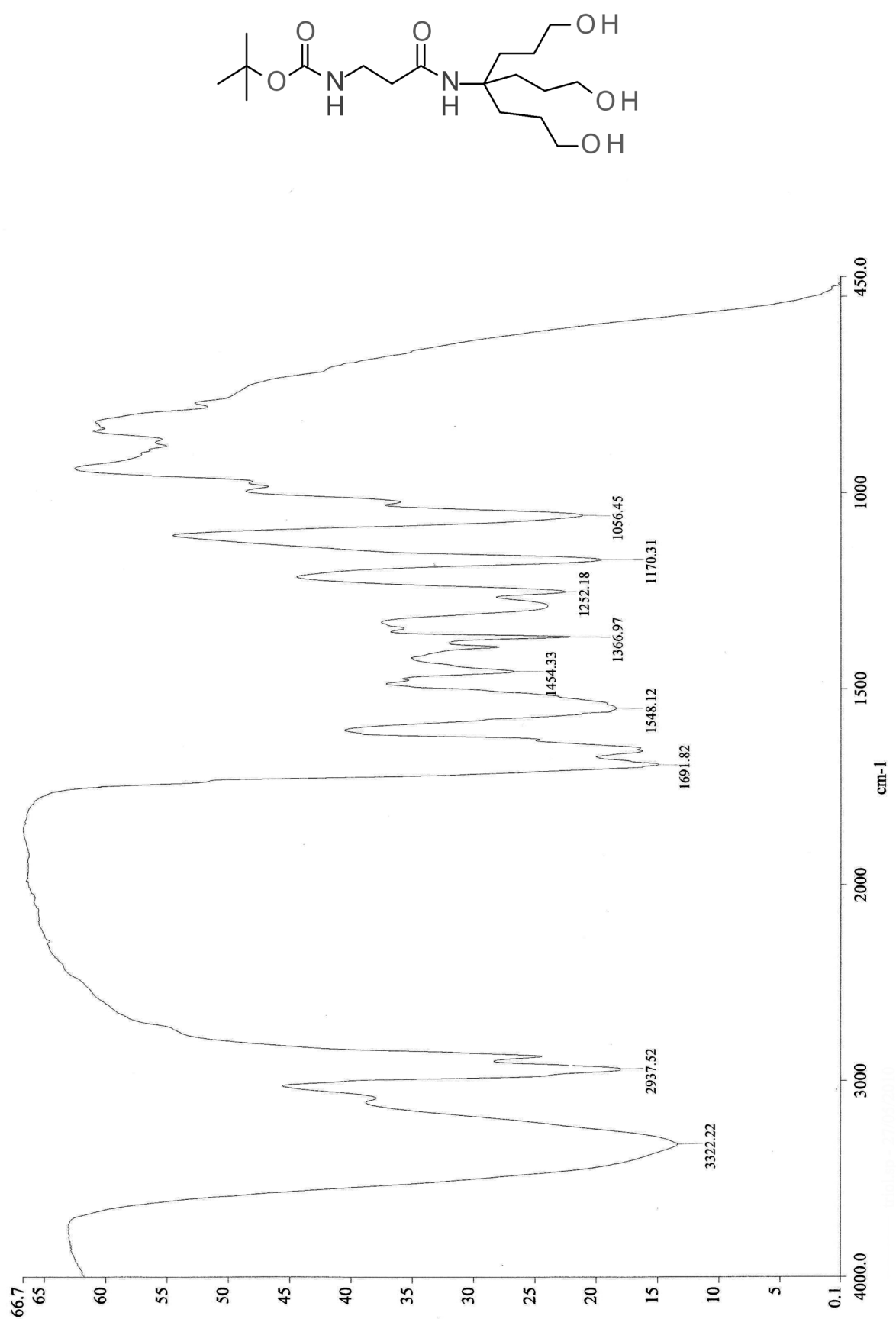














Laboratory at the department of Medicinal Chemistry, King's College London



Erasmus group at the department of Medicinal Chemistry, from left to right:
Dr. Francis Man, Dr. Chiara Buso, Anna Pöschl

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22-06-2010

To whom it may concern

Ms Anna Poeschl

Ms Anna Poeschl joined my research laboratory in February 2010 as an Erasmus student from the University of Vienna. Anna was very keen to become involved in a synthetic project and we arranged for her to work on dendrimer synthesis. Initially, Anna lacked experience of working in a synthetic chemistry laboratory, but soon picked up the skills and towards the end of her 4 month visit, she was able to plan and undertake quite complicated synthetic steps. During her visit she also became familiar with NMR and MS analysis of samples.

Anna's project is based on developing prodrugs of δ -aminolaevulinic acid and of various iron chelators for application in photodynamic therapy of surface tumours. Anna has made major strides in the synthesis of dendrimeric hydroxypyridinones (iron chelators) and has constructed a large segment of a mixed prodrug dendrimer. A PhD student in my lab has now taken over this project, building on Anna's synthetic work.

Anna worked hard and in an efficient manner throughout the 4 month period. She gave an excellent 1 hour oral presentation of her work to the Medicinal Chemists in the department. This was extremely well received. She is a credit to the University of Vienna and would undoubtedly be awarded 1st class honours at King's College London, for the work that she undertook in my laboratory.

Yours sincerely

A handwritten signature in black ink, appearing to be 'R C Hider', with a stylized, cursive script.

R C Hider
Emeritus Professor of Medicinal Chemistry
King's College London