Chemical and Dermatological Aspects of UV-absorbing Compounds

Studies of Photoallergens and Synthesis of a Natural UV-filter

ISABELLA KARLSSON

GÖTEBORGS UNIVERSITET

DOCTORAL THESIS

Submitted in partial fulfillment of the requirements
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Chemical and Dermatological Aspects of UV-absorbing Compounds

Studies of Photoallergens and Synthesis of a Natural UV-filter

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Cover illustration: Proposed mechanism for activation of the commercial UV-filters 4-*tert*-butyl-4'-methoxy dibenzoylmethane and octocrylene by solar radiation resulting in the reaction with skin proteins and the formation of immunogenic complexes.

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Department of Chemistry

University of Gothenburg

SE-412 96 Göteborg

Sweden

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The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' (I found if!) but 'That's funny…'

Isaac Asimov (1920-1992)

ABSTRACT

The sun's UV radiation is necessary for the existence of life on earth. However, too much UV exposure can lead to the development of skin cancer. Therefore, sunscreens are often used by the general population as protection from excessive UV radiation. Unfortunately, many of the chemical UV-filters that are used in sunscreens today have the ability to induce contact and photocontact allergy. In this work two different chemical UV-filters together with the anti-inflammatory drug ketoprofen, all known to induce allergic reactions, have been studied to better understand the reason for these adverse effects. In addition, a synthetic route to the natural UV-filter scytonemin has been developed.

One of the most commonly used UVA-filters today is the well known photoallergen 4-*tert*-butyl-4'-methoxy dibenzoylmethane. We showed that it degrades when irradiated with UV light and that several different photodegradation products are formed. Of particular interest were arylglyoxals and benzils because they were unexplored as potential contact allergens. The benzils were found to be cytotoxic rather than allergenic, whereas the arylglyoxals were found to be strong sensitizers in the murine local lymph node assay (LLNA) used to assess their allergenic potency. Photocontact allergy to dibenzoylmethanes is therefore probably caused by the arylglyoxals that are formed upon photodegradation. Chemical reactivity experiments showed that the arylglyoxals have the ability to form immunogenic complexes via an electrophilic-nucleophilic reaction with the amino acid arginine.

A relatively new UV-filter on the market is octocrylene that has grown in popularity, due to its ability to stabilize other UV-filters such as 4-*tert*-butyl-4'-methoxy dibenzoylmethane. However, recent clinical reports suggest that it is the UV-filter that causes most allergic reactions. Patch and photopatch testing of 172 patients with suspected skin reactions to sunscreens or ketoprofen was performed and 23 of these patients displayed a positive test reaction to octocrylene. Five patients were diagnosed with contact allergy and 18 with photocontact allergy. Notably, many of these patients also displayed a photoinduced reaction to ketoprofen. Without UV radiation, octocrylene was classified as a moderate allergen in the murine LLNA and it was shown to reacted with amines like lysine via a retro-aldol condensation. In presence of UV radiation, octocrylene also reacted with amines but via acyl substitution resulting in a different product outcome than the reaction in the dark. Both the clinical studies and the chemical reactivity experiments thereby indicate that octocrylene has the ability to induce both contact and photocontact allergy.

The apparent photocross-reactivity between octocrylene and ketoprofen observed in the clinical study could not be explained by the previous reactivity studies of octocrylene. Furthermore, according to other clinical reports, photosensitization to ketoprofen also leads to photocontact allergy to many other compounds. Ketoprofen was therefore irradiated in presence of five amino acid analogs and interestingly a reaction between the tryptophan and lysine analogs was substantially enhanced by ketoprofen. We believe that ketoprofen generates singlet molecular oxygen which activates the tryptophan analog that subsequently reacts with the lysine analog. The formation of an immunogenic complex not containing the allergen itself can explain many of the observed photocross allergies between ketoprofen and other structurally different compounds. In theory all compounds that are able to generate singlet molecular oxygen can promote the formation of the same immunogenic complex.

Finally, the first total synthesis of the dimeric alkaloid scytonemin was developed. This natural occurring UV-filter enables the survival of different species of cyanobacteria in areas of intense solar radiation. The planed structure activity studies of scytonemin and derivatives thereof will hopefully lead to the development of a stable UV-filter that does not cause contact or photocontact allergy.

Keywords: Contact allergy, Dibenzoylmethane, Immunogenic complex, Ketoprofen, Local Lymph Node Assay, Octocrylene, Patch testing, Photoallergen, Photocontact Allergy, Photodegradation, Photopatch testing, Photostability, Scytonemin, UV-filter.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numbers I-V. The papers are appended at the end of the thesis and reprints are made with permission from the publishers.

I. Photodegradation of Dibenzoylmethanes: Potential Cause of Photocontact Allergy to Sunscreens
Isabella Karlsson, Lisa Hillerström, Anna-Lena Stenfeldt, Jerker Mårtensson, and Anna Börje.
Chemical Research in Toxicology **22**, 1881-1892 (2009)

II. Clinical and Experimental Studies of Octocrylene's Allergenic Potency
Isabella Karlsson, Katrien Vanden Broecke, Jerker Mårtensson, An Goossens, and Anna Börje.
Contact Dermatitis **64**, 343-352 (2011)

III. Investigation of the Sunscreen Octocrylene's Interaction with Amino Acid Analogs in the Presence of UV radiation
Isabella Karlsson, Jerker Mårtensson, and Anna Börje.
Submitted for publication

IV. Ketoprofen Induced Formation of Amino Acid Photoadducts: Possible Explanation for Photocontact Allergy to Ketoprofen
Isabella Karlsson, Elin Persson, Jerker Mårtensson, and Anna Börje.
Manuscript in preparation

V. Oxidative Coupling as a Biomimetic Approach to the Synthesis of Scytonemin
Andreas Ekebergh, Isabella Karlsson, Rudi Mete, Ye Pan, Anna Börje, and Jerker Mårtensson.
Organic Letters **13**, 4458-4461 (2011)

CONTRIBUTION REPORT

Paper I. Contributed to the formulation of the research problem; performed or supervised the synthesis of test compounds, the development of analytical methods, the chemical reactivity experiments, the LLNA experiments; planed the cell viability experiments; major contribution to interpretation the results; wrote the manuscript.

Paper II. Major contribution to the formulation of the research problem; developed the analytical methods, performed the chemical reactivity experiments and the LLNA experiment; major contribution to interpretation of the results and to the writing of the manuscript.

Paper III. Formulated the research problem; performed all the experimental work; interpreted the results, and wrote the manuscript.

Paper IV. Major contribution to the formulation of the research problem; performed or supervised all the experimental work; interpreted the results, and wrote the manuscript.

Paper V. Minor contribution to the formulation of the research problem; contributed to the experimental work, the interpretation of the results, and to writing of the manuscript.

ABBREVIATIONS

ACD	Allergic contact dermatitis
ACN	Acetonitrile
Arg	Arginine
Cys	Cysteine
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DodSMe	*n*-Dodecyl methyl sulfide
dpm	Disintegrations per minute
EtOH	Ethanol
FDA	US Food and Drug Administration
GC	Gas chromatography
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine
HPLC	High performance liquid chromatography
LDA	Lithium diisopropyl amide
LLNA	Local lymph node assay
Lys	Lysine
MeOH	Methanol
MI	Methylisothiazolinone
MS	Mass spectrometry
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
NSAID	Non-steroidal anti-inflammatory drug
PMS	Phenazine methosulfate
OECD	Organization for Economic Co-operation and Development
PACD	Photoallergic contact dermatitis
PIDA	Phenyliodine diacetate
PBS	Phosphate buffered saline
THF	Tetrahydrofuran
TiO_2	Titanium dioxide
TMANO	Trimethylamine *N*-oxide
Trp	Tryptophan
Tyr	Tyrosine
UV	Ultraviolet
ZnO	Zinc oxide

CONTENTS

1 INTRODUCTION

1.1 Solar UV radiation

Solar radiation has been an important driving force in the development and evolution of life on earth and today sunlight is essential for continued existence of life on the planet. Of the solar radiation that reaches the Earth's surface, the ultraviolet (UV) radiation is of particular importance for the human skin. The UV-region of the electromagnetic spectrum is divided into three different regions: UVC radiation (200-290 nm), UVB radiation (290-320 nm) and UVA radiation (320-400 nm). Of these wavelengths the ozone layer absorbs all UVC radiation as well as large quantities of the UVB radiation. Therefore, the UV radiation that reaches the Earth's surface contains about 5% of UVB and 95% of UVA radiation. (*1*)

1.1.1 Effects of solar UV radiation on human skin

UV radiation has both beneficial and harmful effects on the human body. The most important beneficial effect is the synthesis of vitamin D in the skin. In most people more than 90% of the vitamin D production comes from exposure to solar UVB radiation (*2*). Vitamin D is essential for uptake of calcium and phosphate and also for their integration into bones. On the other hand, it is also well established that unprotected exposure to UV radiation is the main cause for the development of skin cancer. Different wavelengths cause different kinds of DNA lesions. UVB radiation causes direct photochemical damage to DNA, from which gene mutations like thymidine dimers arise. UVA radiation, on the other hand, has indirect effects on DNA via the generation of reactive oxygen species. The most lethal type of skin cancer, cutaneous malignant melanoma, is associated with sporadic intense UV exposure, especially early on in life. However, it has not been clearly identified if melanoma is caused by UVB radiation, UVA radiation or both. In addition, photoageing such as wrinkling and dryness of the skin is caused by exposure to UVA radiation, whereas the shorter wavelengths of UVB are responsible for acute sunburn. (*3*-*6*)

1.2 Sunscreens

In the EU a sunscreen is defined as a preparation intended to be placed on the skin with the purpose to protect it from UV radiation by absorbing, scattering or reflecting the radiation (*7*). Sunscreens have been used since 1928 and sunscreen application is today one of the most common strategies to protect one-self from the numerous harmful effects of excessive sunlight. The active sunscreen compounds are divided into inorganic (also called physical) and organic (also called chemical) UV-filters. Physical UV-filters work by reflection, absorption and scattering of both UV and visible light. By far, the most common physical UV-filters are titanium dioxide (TiO_2) and zinc oxide (ZnO). Chemical UV-filters act only by absorbing the UV radiation and are therefore composed of conjugated aromatic systems. The absorption of the UV radiation excites the UV-filter to a higher energy level, and in the ideal case it returns unchanged to the ground state and releases the excess energy in the form of heat. The chemical UV-filters are divided into UVB and UVA-filters; see Figure 1.1 for the most common classes of UVB and UVA-filters. Since sunscreens have to provide

protection in the complete UVB and UVA spectrums (*7*) they often contain a combination of different UV-filters.

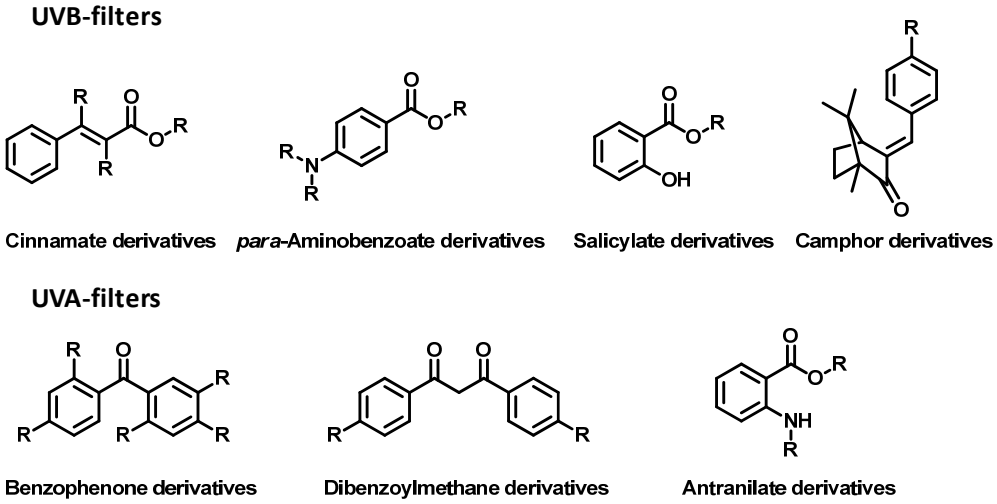


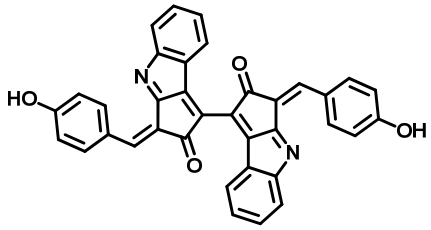
Figure 1.1. General structures of the most common classes of chemical UVB and UVA-filters.

Presently there are 28 approved UV-filters in the EU. Of these 28, 27 are chemical UV-filters and only one, TiO_2, is a physical UV-filter (*8*). In the US there are only 17 approved UV-filters of which 15 are chemical UV-filters and two, TiO_2 and ZnO, are physical UV-filters. A plausible reason for the big difference in how many UV-filters that are approved is that in the EU sunscreen agents are classified as cosmetic products (*8*), whereas they are classified as over-the-counter-drugs by the US Food and Drug Administration (FDA) (*9*).

Although the use of sunscreen has been shown to decrease the risk of developing squamous cell carcinoma and actinic keratoses, it is still a matter of debate whether sunscreen use has any impact on the risk of developing cutaneous malignant melanoma and basal cell carcinoma (*3-5, 10, 11*). Today chemical UV-filters are not only used in sunscreens but also in cosmetics and toiletries (*12*), probably partly because the users want products that also protect them from solar radiation. However, the UV-filters are probably also included in the formulations to protect other components in the product from degradation when subjected to solar radiation. The increased use of UV-filters in different skin care products, together with the population's rising awareness of the detrimental effects of UV radiation, has increased people's exposure to chemical UV-filters. Unfortunately this elevated exposure has led to an increase in unwanted side effects such as contact and photocontact allergy to chemical UV-filters (*5, 13*).

1.3 The Natural UV-filter Scytonemin

Scytonemin (Figure 1.2) is a UV-screening pigment that is commonly produced in populations of sheathed cyanobacteria that live in different habitats and geographic locations, but always in environments where solar radiation is very intense (*14*). The yellow-green pigment scytonemin was first reported by Nägeli as early as 1849. However, the structure was unknown for over 100 years until 1993 when a complete elucidation of the chemical structure was provided by Proteau et al (*15*).



Scytonemin

Figure 1.2. Structure of the natural UV-filter Scytonemin found in a wide range of different cyanobacteria.

Scytonemin is a lipid soluble alkaloid that is synthesized in response to UVA radiation and accumulates within the extracellular sheaths of cyanobacteria. The organisms are thereby protected from cell damage by this natural UV-filter that absorbs the harmful solar radiation (*14-16*). Scytonemin absorbs mostly in the UVA (325-425 nm, λ_{max} = 370 nm) and UVC region (λ_{max} = 250 nm), but it also absorbs substantially in the UVB region (280-320 nm) (*15*). In addition to scytonemin's important function as a sunscreen it also possesses anti-proliferative (*17*), anti-inflammatory (*18*) and antioxidant properties (*19*).

1.4 Allergic Contact Dermatitis

In the western world 15-20% of the population is allergic to one or more compounds in their environment (*20, 21*). Allergic contact dermatitis (ACD), which is the clinical manifestation of contact allergy, is an undesired consequence of our immune system. ACD is caused by a wide range of chemicals upon skin contact and the most common contact allergens are metals, fragrances and preservatives (*22-24*). The immunological memory created in the development of contact allergy is lifelong and since there is no cure for ACD the only way to prevent development of eczema is to avoid the allergenic compound.

1.4.1 Immunological mechanism

ACD has two main phases: sensitization and elicitation. Sensitization is the induction phase which results in an immunological memory (Figure 1.3). The elicitation phase takes place upon renewed contact with the same chemical, which then results in an inflammatory reaction (Figure 1.3). ACD is regarded as a T-lymphocyte mediated type IV hypersensitivity reaction and is thought to be caused by reactive chemicals (haptens) of low molecular weight (<1000 g/mol) and appropriate lipophilicity (Log*P* ~ 2) that are able to penetrate into epidermis. The haptens themselves are too small to be recognized by the immune system, so in order to cause an allergic response they need to react with macromolecules in the skin (*25*). The macromolecules are usually considered to be proteins and the haptenated proteins are referred to as immunogenic hapten-protein complexes.

Langerhans cells are professional antigen-presenting dendritic cells present in the skin. In the sensitization phase, the Langerhans cells are activated by the immunogenic hapten-protein complex and migrate to the local lymph nodes where they present the processed immunogenic hapten-protein complex (the antigen) to naïve T-cells. Recognition of the antigen by the naïve T-cells results in the formation of antigen specific effector and memory T-cell clones that start to circulate the blood and lymphatic system. (*26*)

The elicitation phase starts with re-exposure to the same hapten, which again results in the formation and processing of the immunogenic hapten-protein complex in epidermis. However, this time the memory T-cells formed in the sensitization phase are recruited to the site of contact, and the interaction between T-cells and antigen-presenting cells takes place directly in the epidermis. This interaction initializes the inflammatory process that leads to development of eczema at the site of exposure. (*26*)

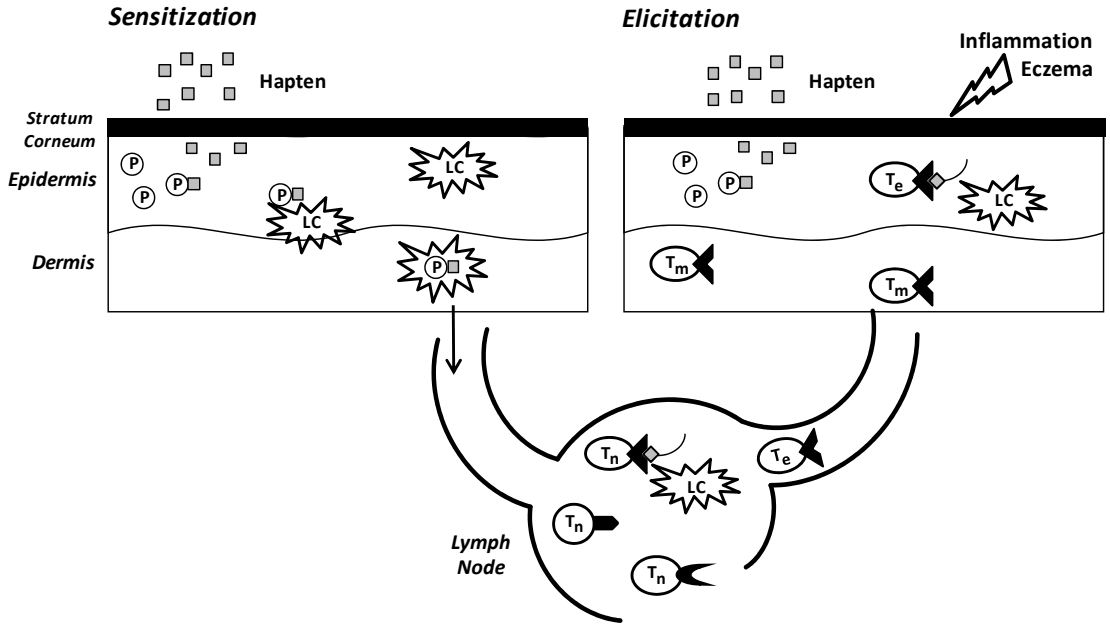


Figure 1.3. An overview of the immunological mechanism in allergic contact dermatitis. In the sensitization phase the hapten penetrates the skin and reacts with a protein (P), thus forming a hapten-protein complex. This complex activates the Langerhans cells (LC) that migrate to the lymph node where they present the antigen to naïve T-cells (T_n). Recognition of the antigen by a naïve T-cell specific for the presented antigen activates the T-cell that starts to proliferate and differentiate to antigen specific effector (T_e) and memory (T_m) T-cell clones that circulate the blood and lymphatic system. Upon re-exposure to the same hapten (elicitation) the memory T-cells are activated in the skin by antigen presenting cells which leads to an inflammatory reaction.

1.4.2 Hapten-protein interactions

There are different ways in which a hapten can react with a protein and form an immunogenic hapten-protein complex. Most haptens are form immunogenic hapten-protein complexes via an electrophilic-nucleophilic interaction with the nucleophilic moieties of the amino acid side chains in skin proteins. An electrophile is an electron-deficient species that can form a bond by accepting an electron pair from the electron-rich nucleophile. The nucleophiles of primary interest are thiols (-SH) and primary amines ($-NH_2$) present in cysteine residues and lysine residues, respectively. The electrophilic nature of a molecule is

therefore considered to be a good predictor for its sensitizing capacity (*27*). The most common electrophilic-nucleophilic reactions, in the formation of immunogenic hapten-protein complexes, are Michael additions, S_N2 reactions and nucleophilic addition to carbonyls (Figure 1.4). Metals, such as nickel, cobalt and chromium, are a group of haptens that give immunogenic hapten-protein complexes via a different mechanism. These metals yield stable positively charged ions that coordinate to the electron-rich ligands of proteins, which thereby result in highly stable complexes (*25*). A third mechanism, by which immunogenic hapten-protein complexes can be formed, is via a radical reaction. Urushiols (*28, 29*) and hydroperoxides (*30, 31*) are classes of compounds that have been shown to react with proteins in a radical mechanism.

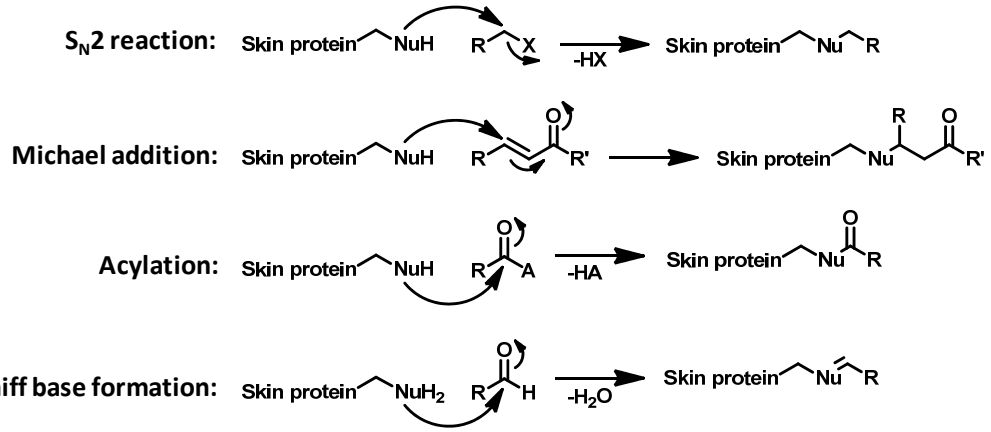


Figure 1.4. The electrophilic-nucleophilic reactions most commonly involved in the formation of allergenic hapten-protein complexes. X = Cl, Br or I; R = R' = alkyl, aryl, or H; A = good leaving group.

1.4.3 Cross-reactivity

Cross-reactivity is when sensitization caused by one allergen automatically leads to contact allergy to another allergen, although the person or animal has never been subjected to the later; for example, corticosteroids are a class of compounds that are known to cross-react (*32*). The general belief is that in most cases it is the part of the protein that contains the hapten that is recognized by a T cell. According to this hypothesis allergenic compounds have to form the same or very similar immunogenic complex in order for them to cross-react. This theory is further supported by cross-reactivity studies in both patients and animals (*32-35*) in which only structurally very similar compounds had the ability to cross-react.

1.5 Photoallergic Contact Dermatitis

When a molecule absorbs radiation it can lead to the formation of the electronically excited form of the molecule. This excited-state species can display different chemical properties compared to the ground-state species. There are different ways in which the excited-state molecule can lose the excess energy and go back to its ground-state. Dissociation is one common pathway of the excited state species to lose the energy. In this case the absorbed energy is enough the break a bond, which leads to fragmentation of the molecule. This process is often called *photolysis*, which is Greek for 'splitting by light'. Another term that is commonly used is *photodegradation* and the new molecules that are formed are often

referred to as photodegradation products. Some excited-state species can take part in reactions that are not possible for the ground-state species. This could be either because the excess energy can be used to overcome an activation barrier or because the particular electronic arrangement of the excited-state molecule enables the reaction. One such example in which some electronically excited molecules get rid of the excess energy is by isomerization, for example *E-Z* isomerization of an alkene. Energy transfer from the excited-state molecule that first absorbed the energy to another molecule is also a possible pathway. Radiative loss of the energy is another possible route for losing the excess energy. (*36*)

Photoallergic contact dermatitis (PACD) arises when a compound after absorption of light forms a hapten or an immunogenic complex that causes an allergic reaction. The compound itself may be non-allergenic, or an already existing contact allergen as in the case of combined contact and photocontact allergic reactions. The most frequently encountered photoallergens are chemical UV-filters and non-steroidal anti-inflammatory drugs (NSAIDs) (*13, 37-40*). Photochemical changes are mainly induced by UVA radiation and less frequently by UVB or visible light (*39, 40*). Photoallergens are compounds that form haptens or immunogenic complexes once activated by light. The precise mechanism for the formation of an immunogenic complex in PACD is not fully understood. However, theoretically three different pathways are possible (*36*):

i. Fragmentation of the molecule gives a photodegradation product that serves as a hapten.
ii. The excited state molecule reacts with a protein, thus forming an immunogenic complex, either by overcoming an activation barrier or as a consequence of the new electronic arrangement.
iii. The excited state molecule can transfer the energy to another molecule, like a skin protein, which then becomes modified in such a way that the immune system will think of it as non-self.

The symptoms of PACD are eczematous reactions identical to other forms of ACD. The eczema usually occurs on the areas that have been exposed to light. However, it may develop at a different place depending on which part of the body that is exposed. The duration of the symptoms after ending application of a photoallergen varies with different substances. For sunscreens the duration is usually less than 4 days, whereas it for NSAIDs can last for several weeks after the last application (*39*).

1.5.1 Dibenzoylmethanes

In the eighties and early nineties one of the most commonly used UVA-filters was 4-isopropyldibenzoylmethane (Figure 1.5). However, it was shown to cause allergic reactions and was voluntarily removed from the market in 1993 and replaced with a structurally very similar compound, 4-*tert*-butyl-4'-methoxy dibenzoylmethane (Figure 1.5) (*41*). Today 4-*tert*-butyl-4'-methoxy dibenzoylmethane is the only approved dibenzoylmethane in EU as well as in the US. It is well known that 4-*tert*-butyl-4'-methoxy dibenzoylmethane photodegrades when irradiated (*42-47*) but despite that it is one of the most commonly used UVA-filters and it is allowed in concentrations up to 5% in EU (*8*) and up to 3% in the US (*9*).

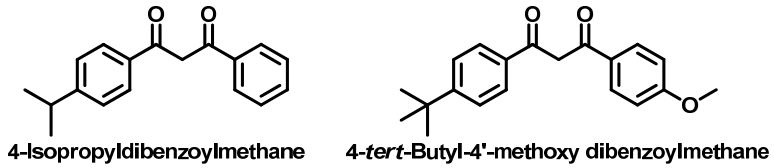
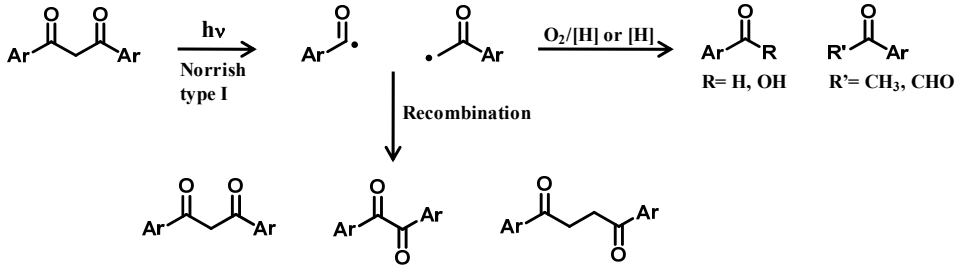


Figure 1.5. The compound to the left, 4-isopropyldibenzoylmethane, is the dibenzoylmethane that was used in sunscreens in the eighties and early nineties and the compound to the right, 4-*tert*-butyl-4'-methoxy dibenzoylmethane, that is the dibenzoylmethane that is used in sunscreens today.

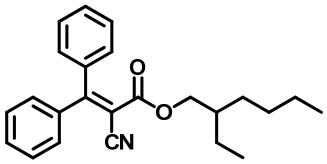
There are a number of clinical reports on photocontact allergy caused by 4-*tert*-butyl-4'-methoxy dibenzoylmethane and it therefore does not seem as if 4-*tert*-butyl-4'-methoxy dibenzoylmethane would be significantly less sensitizing than 4-isopropyldibenzoylmethane. Furthermore, Schwack and Rudolph showed in 1995 that both of these dibenzoylmethanes photodegrade via a Norrish type I radical mechanism (Scheme 1.1) and that the same type of stable photoproducts are formed (*45*).



Scheme 1.1. Photodegradation of dibenzoylmethanes via a Norris type I radical mechanism (*45*).

1.5.2 Octocrylene

Octocrylene is a chemical UV-filter that belongs to the cinnamate family (Figure 1.6) and provides protection against UVB and short UVA wavelengths (*4*). It is a hydrophobic compound with a LogP of 6.9 (*48*) and it is considered to be photostable (*42*). It has also been shown to stabilize other UV-filters, such as 4-*tert*-butyl-4'-methoxy dibenzoylmethane and is therefore included in many skin products as a photostabilizer (*42-44*, *49-51*).



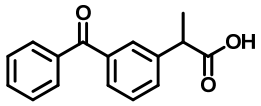
Octocrylene

Figure 1.6. Structure of the chemical UVB-filter octocrylene.

These attractive properties have contributed to its widespread use in sunscreens and cosmetic preparations during the last 10 years (*52, 53*) and it is approved by EU and FDA at concentrations up to 10% (*8*, *9*). The first cases of contact and photocontact allergy were not reported until 2003 by Carrotte-Lefebvere et al (*54*). However, the reports of contact allergy and photocontact allergy to octcorylene are currently increasing (*52-56*) and it has even been suggested to be the chemical UV-filter that causes most allergic and photoallergic reactions (*38*).

1.5.3 Ketoprofen

Ketoprofen, 2-(3-benzoylphenyl)propionic acid (Figure 1.7), is an NSAID that is used both topically and systematically. It is widely administered topically for treatment of musculoskeletal pain. It can be found in different topical formulations such as gels, lotions, creams, ointment and plasters. These ketoprofen containing topical formulations have previously been available over-the-counter in Europe, but because of the increasing number of clinical reports on photo induced adverse skin reactions from topically administered ketoprofen, the European Commission has decided to make it a prescription drug (*57*). In the US there are no topical formulations containing ketoprofen that are approved by the FDA. However, there are a few topical formulations containing ketoprofen that are in clinical trials (*58, 59*).



Ketoprofen

Figure 1.7. Structure of the non-steroidal anti-inflammatory drug ketoprofen.

The first report on contact allergy caused by ketoprofen came in 1983 (*60, 61*) and the first reported case of photocontact allergy in 1985 (*62*). Today it is common with clinical reports on contact allergy and especially photocontact allergy caused by ketoprofen (*38, 39, 53, 56*), and the symptoms are often severe and can in many cases be persistent (*38, 56, 63*). Furthermore, patients with photocontact allergy to ketoprofen often show positive photoinduced reactions to a number of other allergens such as tiaprofenic acid, suprofen, fentichlor, etofenamate, fenofibrate, and benzophenone derivatives (*64-66*). More recently, studies indicating a photocross reactivity between ketoprofen and the UV-filter octocrylene have been published (*53, 56*).

2 AIMS OF THE STUDIES

With the overall goal to gaining more knowledge regarding the chemical and photochemical properties of photoallergens, the objectives of this thesis were:

1. To study the photostability of dibenzoylmethane derivatives, and to investigate the sensitizing capacity of the dibenzoylmethanes and their degradation products to better understand the chemistry behind their photoallergenic properties (Paper I).

2. To study contact and photocontact allergy to octocrylene, in patients with adverse skin reactions to sunscreens and/ or ketoprofen (Paper II).

3. To investigate octocrylene's sensitizing capacity and its chemical reactivity toward amino acids to provide knowledge regarding its ability to induce contact allergy (Paper II).

4. To study octocrylene's photostability and its excited state reactivity toward different amino acid analogs in order to understand the reason for its photoallergenic properties (Paper III).

5. To investigate ketoprofen's photostability and effect on amino acid adduct formation in presence of UV radiation with the purpose of gaining a deeper knowledge of ketoprofen's ability to induce photocontact allergy (Paper IV).

6. To develop a synthetic route to the natural UV-filter scytonemin so that in the future a photostable chemical UV-filter that does not cause adverse skin reactions can be developed (Paper V).

3 METHODS AND TECHNIQUES

3.1 Patch and Photopatch Testing

In Paper II, patients with suspected skin reactions to sunscreens or ketoprofen were patch and photopatch tested at the Department of Dermatology, University Hospital Saint-Raphaël, Katholieke Universiteit, in Leuven in Belgium. In total 172 patients were patch tested and 90 of these were also photopatch tested. The patients were tested with chemical UV-filters including octocrylene (10% in petrolatum) and from May 2008 the patients were patch and photopatch tested with the European baseline photopatch series, which also included the NSAID ketoprofen (1% in petrolatum). In the patch test the compounds were applied, in small test chambers, on one side of the patients back and left under occlusion for two days before removal. The reactions were then evaluated directly after removal on day 2 and again on day 4 and day 7. In the photopatch test, chambers with the different test substances were similarly applied on the opposite side of the patients back and removed after two days. After removal, the part of the patients back where the photopatches had been situated was irradiated with UVA (5 J/cm^2). The photopatch tests were then evaluated immediately after irradiation on day 2 and then again on day 4 and day 7 (2 and 5 days after irradiation, respectively). The evaluation was done according to the International Contact Dermatitis Research Group Recommendations (*67*): +++ = vesicles, papules, erythema and infiltrate; ++ = papules, erythema and infiltrate; + = erythema and infiltrate; - = no reaction.

3.2 Local Lymph Node Assay

The murine local lymph node assay (LLNA) that has been used in Papers I and II, is today considered to be the golden standard in assessment of sensitizing potency. Furthermore, this method is accepted and recommended by the Organization for Economic Co-operation and Development (OECD) (*68, 69*). In the LLNA experiments performed in these papers, six groups with three female CBA/CA mice in each group were used. Acetone/ olive oil (4:1 v/ v) was used as vehicle to dissolve the test compounds. In each experiment one group was used as a control group and received the vehicle only, whereas the other five groups received different concentrations of the test substance. Twenty-five micro-liters of a solution of the test compound, or the vehicle alone, was applied to the dorsum of the ears of the mice for three consecutive days. Five days after the first treatment, the mice were injected intravenously with 250 µL of phosphate buffered saline (PBS) containing 20 µL of [^{3}H]-thymidine. Five hours after the injection, the mice were euthanized and the draining lymph nodes were excised. All lymph nodes from the same group were pooled and a single-cell suspension was prepared and the [^{3}H]-thymidine incorporation was measured by β-scintillation counting. A schematic overview of the LLNA procedure can be seen in Figure 3.1.

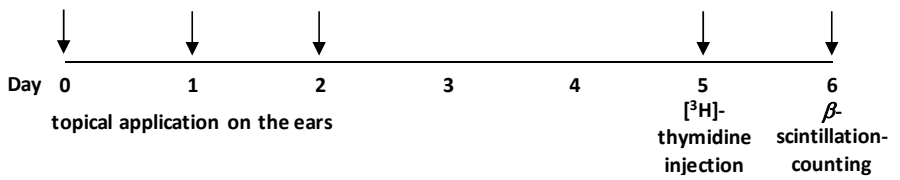


Figure 3.1. Schematic overview showing the timeline of the local lymph node assay (LLNA).

The result from the LLNA is expressed as the disintegrations per minute (dpm) divided by the number of lymph nodes for each experimental group and as a stimulation index (SI). The SI is defined as the ratio between the dpm/lymph node for the test group (*dpmX*/*nX*) and the control group (*dpmY*/*nY*) (Eq. 3.1). If the test compound in any concentration gives an SI above 3 the compound is classified as a sensitizer. The estimated concentration required to cause an SI greater than 3 (EC3) is calculated by linear interpolation (Eq. 3.2), in which *conc A* is the test concentration that gives an SI immediately above 3 (*SIa)* and *conc B* is the test concentration that gives an SI immediately below 3 (*SIb)* (*70*). In this work the sensitizing potency of the test compounds has been classified according to the proposal made by Kimber et al in 2003 (*71*): < 0.1%, extreme; ≥ 0.1% to < 1%, strong; ≥ 1% to < 10%, moderate; and ≥ 10% to 100%, weak.

$$\text{SI} = \frac{dpmX/nX}{dpmY/nY} \tag{3.1}$$

$$\text{EC3} = conc\ B + \frac{(conc\ A - conc\ B)(3 - SIb)}{SIa - SIb} \tag{3.2}$$

3.3 Cell Viability Assay

In Paper I a cell viability assay was used to measure the cytotoxicity of benzils **4.3a**, **4.3c-e**. In this non-radioactive cell proliferation assay, solution of the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)], and an electron coupling reagent [phenazine methosulfate (PMS)] are used *(72-74*). The MTS compound is reduced into an aqueous soluble formazan product by dehydrogenase enzymes found in metabolically active cells (Figure 3.2). The absorbance of the formazan product, which can be measured directly by UV-vis spectroscopy from 96-well plates, is proportional to the number of living cells.

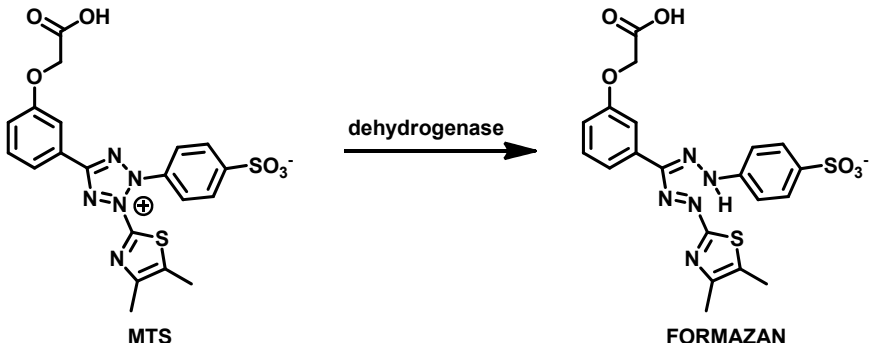


Figure 3.2. Structures of the MTS tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) and the formazan product formed by metabolically active cells.

3.3.1 Cell culture

A human U937 monoblastoid cell line was used in the cell viability assay performed in Paper I. The cells were cultured in a 5% CO_2 atmosphere at 37 °C in phenol red-free RPMI-1640 medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 14 mM glucose, 100 U/mL pencillin,

100 µg streptomycin, and 10% fetal bovine serum. Cells were harvested at the ninth passage for usage in the MTS assay.

3.3.2 Experimental procedure

Cells were seeded into 96-well plates at a density of 5000 cells/well in 0.1 mL of RPMI- 1640 medium with all the additives used during cell culture except for the antibiotics. The cells were incubated in a 5% CO_2 atmosphere at 37 °C for 3 hours before the addition of test compounds. For benzils **4.3c** and **4.3e** stock solutions containing 100 mM in ethanol (EtOH) were prepared. These were then diluted with EtOH to 25 and 12.5 mM. A volume of 1 µL was finally added to the wells, which gave final concentrations of 1, 0.25, and 0.125 mM for benzils **4.3d** and **4.3e**. Due to solubility problems, a 50 mM stock solution of benzil **4.3a** in EtOH was prepared that was further diluted with EtOH to 12.5 and 6.25 mM. A volume of 2 µL was then added to the wells, resulting in final concentrations of 1, 0.25, and 0.125 mM for benzil **4.3a**. No more than 25 mM of benzil **4.3d** could be dissolved, even when a mixture of dimethyl sulfoxide (DMSO)/ EtOH 1:10 was used. The stock solution was diluted with EtOH to 12.5 and 6.25 mM. A volume of 2 µL was then added to the wells, which gave the following final concentrations for benzil **4.3d**: 0.5 (0.2% DMSO and 1.8% EtOH), 0.25 (0.1% DMSO and 1.9% EtOH), and 0.125 mM (0.05% DMSO and 1.95% EtOH). Copper sulfate, dissolved in medium, was used as positive control in two different concentrations: 0.02 and 0.2 mM, and medium with the different solvent compositions, used to dissolve the benzils, were used as negative controls. Triplicates were made for all test compounds, as well as for both positive and negative controls. The plate, with the test compounds and the controls, was incubated in a 5% CO_2 atmosphere at 37 °C for 24 hours. Twenty micro liters of the MTS/ PMS reagents, which had been mixed in a 20:1 ratio, were then added to the wells and the plate was incubated for another 2 hours, after which the absorbance at 492 nm was measured. After the measurement, tryphan blue was added and the cells status was studied visually by light microscopy.

3.4 Photolysis Experiments

Photostability is a crucial property for a sunscreen, but unfortunately not all UV-filters remain stable during UV irradiation. In order to understand the photochemical mechanisms behind sun-induced allergic contact dermatitis, photolysis experiments could be used to establish which photochemical degradation products can be formed from a photoallergen. Therefore, in Papers I, III, and IV photolysis experiments were performed in a falling film photoreactor with forced liquid circulation (*75*).

3.4.1 Experimental setup

A motor driven impeller forces the reaction liquid up through a riser pipe which then flows down evenly in a thin film on the inside of the wall of a wide vertical glass tube. The outside of the glass tube is cooled with water to keep the temperature between 20 °C and 40 °C. As the reaction liquid flows down the tube, in a thin film, it is irradiated by a water cooled, medium pressure mercury UV lamp, positioned inside the glass tube; the radiant power of the lamp, at different wavelengths, can be found in Table 3.1. The distance from the lamp and the thin film liquid varies from 0.04 to 0.1 meter, and all parts between them are made of quartz glass. After the reaction liquid has passed the wide vertical glass tube, where it has been irradiated, it is collected in a vessel, from which samples can be withdrawn during the

experiment. The vessel also contains the impeller, which causes the circulation of reaction liquid. In addition, a gas inlet is connected to the top of the vessel so the appropriate atmosphere can be applied in the experiment.

Table 3.1. The radiant power (Φ) at 200-600 nm and 700 W for the UV lamp in the photoreactor.[1]

λ (nm)	Φ (W) at 700 W
200-280	75
280-315	45
315-400	147
400-600	122
Total radiant power	389

[1]The lamp is a 700 W TQ 718, Z4 doped, medium pressure mercury lamp, purchased from Heraeus Noblelight GmbH.

3.4.2 Experimental procedure

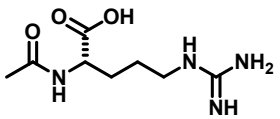
Solutions of 10 mM of test compound or compounds were dissolved in 350-370 mL of EtOH or cyclohexane, except for the dibenzoylmethanes for which only 2 mM could be dissolved in that volume. Synthetic air was used as atmosphere in all experiments. Samples of approximately 4 mL were removed from the photoreactor at specific time intervals and diluted twenty times with acetonitrile (ACN) before analysis with high performance liquid chromatography/mass spectrometry (HPLC/MS) or with either dichloromethane (DCM) or *n*-hexane before analysis with gas chromatography/mass spectrometry (GC/MS). Standard curves for the different photolysed compounds and photodegradation products were used to quantify the amounts of the different compounds.

3.5 Chemical and Photochemical Reactivity Experiments

In both the sensitization and elicitation phase a hapten is believed to interact with a macromolecule in the skin. Therefore, chemical and photochemical reactivity experiments using amino acids and/or model nucleophiles were performed in Papers I – IV to mimic this interaction with skin proteins.

3.5.1 Chemical reactivity experiments with arylglyoxals

In Paper I chemical reactivity experiments of arylglyoxals **4.2b**-**d** toward the protected amino acid arginine (Figure 3.3) were performed. In all experiments quantification was made with the HPLC/MS or GC/MS using standard curves.



***N*$_\alpha$-Acetyl-L-arginine**

Figure 3.3. Structure of the nucleophile *N*$_\alpha$-acetyl-L-arginine.

A 1 mL solution of 20 mM arylglyoxals in DMSO/ phosphate buffer (pH 7.0) 1:4 was added dropwise to a 1 mL stirred solution of 20 mM *N*$_\alpha$-acetyl-L-arginine and 20 mM internal standard (2-nitrotoluene) in DMSO/ phosphate buffer pH 7.0 (1:4). The reaction mixture was stirred in room temperature and aliquots of 100 µL were withdrawn after 15, 45, 75, 105,

135, 165 and 205 minutes. These samples were diluted ten times with milli-Q water and analyzed with HPLC/MS.

3.5.2 Chemical reactivity experiments with octocrylene

In Paper II chemical reactivity experiments of octocrylene toward the nucleophiles benzylamine, N_α-acetyl-L-lysine-methyl ester and N_α-acetyl-L-cysteine-methyl ester were performed; structures of the nucleophiles can be seen in Figure 3.4.

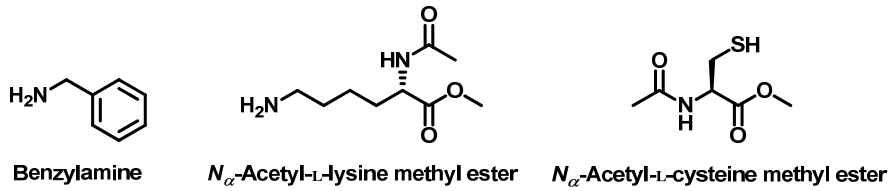


Figure 3.4. Structure of the nucleophiles benzylamine, N_α-acetyl-L-lysine-methyl ester and N_α-acetyl-L-cysteine-methyl ester.

A 2 mL solution containing 10 mM octocrylene, 10 mM internal standard (dibutylphtalate) and 100 mM of nucleophile in EtOH or ACN/ carbonate buffer pH 10 (3:1) was stirred at room temperature and aliquots of 10 µL were removed at specific time intervals. The removed samples were diluted to 200 µL with either ACN/ carbonate buffer pH 10 (3:1) or ACN/ carbonate buffer pH 10/ EtOH (27:9:2) prior to HPLC/MS analysis.

3.5.3 Photochemical reactivity experiments

In Papers III and IV photochemical reactivity experiments were performed with octocrylene, ketoprofen or benzophenone-3, together with analogs of the amino acids: tyrosine (Tyr), tryptophan (Trp), cysteine (Cys), lysine (Lys) and histidine (His); structures can be seen in Figure 3.5.

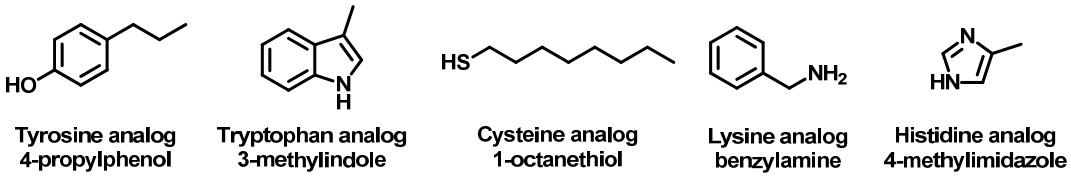


Figure 3.5. Structure of the analogs for the amino acids: tyrosine, tryptophan, cysteine, lysine, and histidine.

Photolysis of solutions containing 10 mM of test compound and 10 mM of one or several of the amino acid analogs was performed according to the procedure described in section 3.4.2.

4 STUDIES OF DIBENZOYLMETHANES (PAPER I)

The only difference between 4-*tert*-butyl-4'-methoxy dibenzoylmethane (**4.1a**) and 4-isopropyldibenzoylmethane (**4.1b**) is that of their *para* substituents (Figure 4.1). In this study we wanted to investigate if the replacement of dibenzoylmethane **4.1b** with dibenzoylmethane **4.1a** in skin care products would give a less photosensitizing compound. Schwack and Rudolph (*45*) had previously shown that both of these dibenzoylmethanes photodegrade via a Norrish type I radical mechanism (Scheme 1.1, Section 1.5.1). However, the sensitizing capacity of many of the formed photodegradation products had not previously been studied. First we wanted to repeat the photodegradation experiment of **4.1a** in order to establish the relative amounts formed of each photoproduct, and secondly we wanted to study the sensitizing potency of both the two dibenzoylmethanes and of the photoproducts that had not previously been tested.

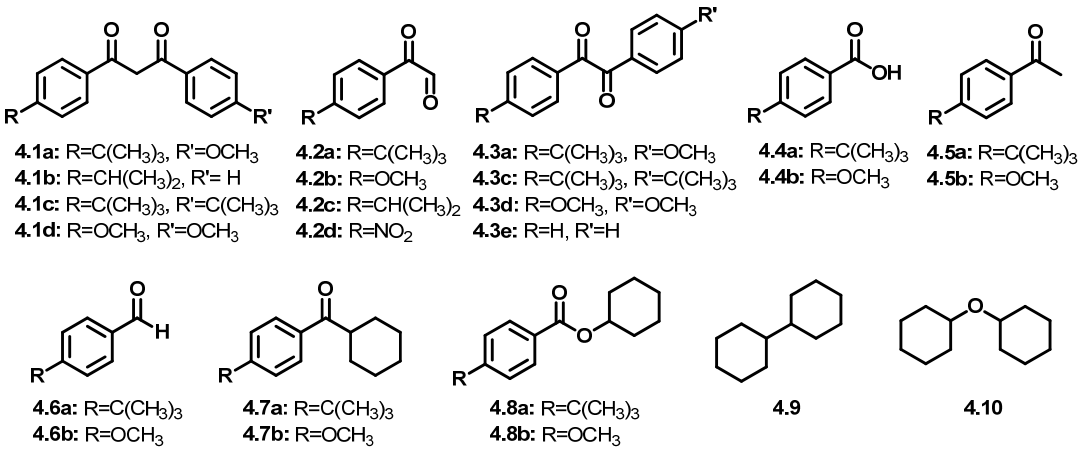
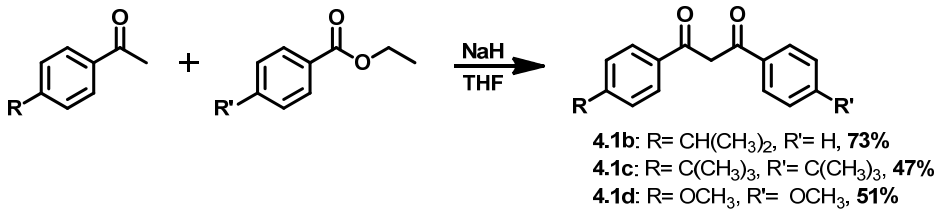


Figure 4.1. Structures of compounds discussed in this study.

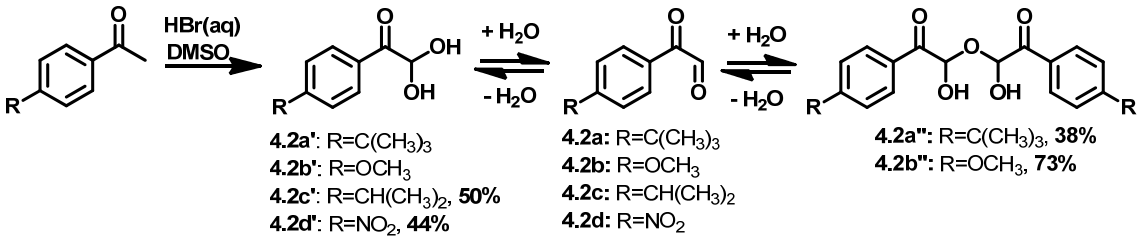
4.1 Synthesis

The unsymmetrical dibenzoylmethane **4.1a** was commercially available, but the other three dibenzoylmethanes **4.1b**-**d** had to be synthesized. A literature procedure (*76*) was used to obtain compounds **4.1b**-**d** in moderate yields via a mixed Claisen condensation of their corresponding acetophenones and ethylbenzoates (Scheme 4.1).



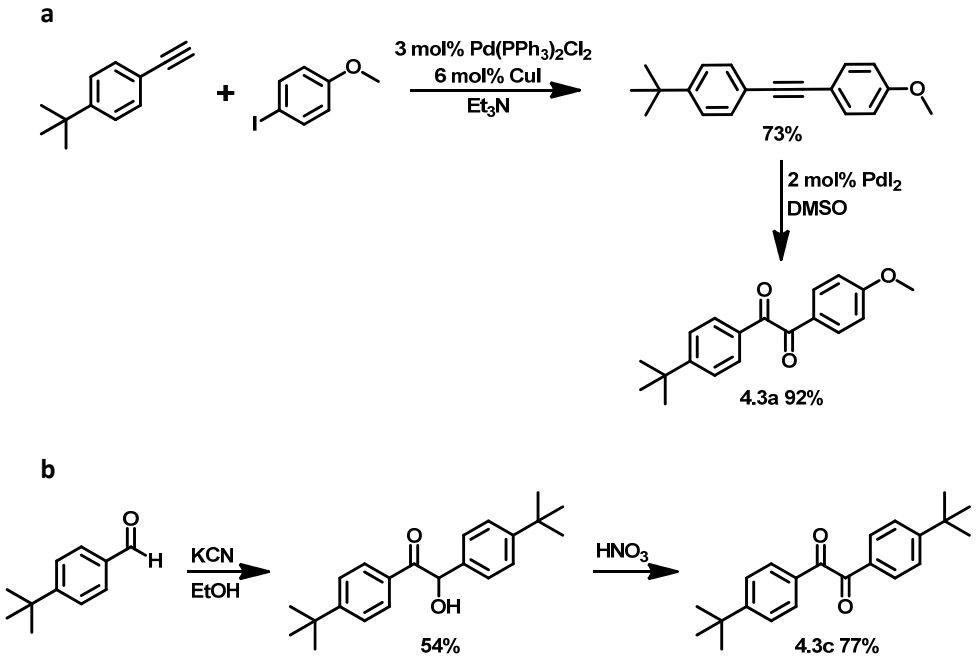
Scheme 4.1. Synthetic route to the studied dibenzoylmethanes that had to be synthesized **4.1b-d**.

The arylglyoxals **4.2a**-**d** were also obtained in moderate yields (Scheme 4.2). However, they were synthesised as their hemiacetal dimers (**4.2a''** and **4.2b''**) or hydrates (**4.2c'** and **4.2d'**) from their corresponding acetophenones via an oxidation with aqueous HBr in DMSO (Scheme 4.2) (*77*). In the presence of water, an equilibrium between the glyoxal and its hydrate will rapidly be established (Scheme 4.2) (*78, 79*), and since the hydrates have been found to be more stable than the glyoxals (*78*), the hydrates of compound **4.2a**-**d** were our prime target for the synthesis. However, arylglyoxals **4.2a** and **4.2b** did not crystallize in their hydrate form; instead these were found to crystallize in a dimeric hemiacetal form. Furthermore, the *tert*-butyl analogue **4.2a''** co-crystallized with a small fraction of solvent molecules. Both the formation of dimeric hemiacetals and co-crystallization with solvent have been reported earlier for other arylglyoxals (*80, 81*). Fortunately, the dimeric hemiacetal form, similarly to the hydrate, is in equilibrium with the corresponding glyoxal in solution (Scheme 4.2) (*82*). This was confirmed by HPLC/MS and NMR studies. The dimeric hemiacetal are stable in DMSO-d_6, but no dimers could be detected in D_2O/DMSO-d_6 (1:1) with the NMR spectrometer, which shows that they in this solvent system hydrolyze to the corresponding hydrates instantly. This is also in accordance with the results obtained from the chemical reactivity experiment toward arginine in which no difference in reactivity can be seen between the hydrates **4.2c'** and **4.2d'** and the hemiacetal **4.2b''** (vide infra). Thus, both the hydrate form and the hemiacetal dimer can be considered to be arylglyoxal equivalents. However, the hydrate corresponds to one unit of arylglyoxal and the hemiacetal dimer to two units. In the remainder of this thesis, these equilibrium mixtures will be referred to as arylglyoxals **4.2a**-**d**.



Scheme 4.2. Synthetic route and yields to the dimeric hemiacetals and hydrates of the arylglyoxals **4.2a-b**.

Both benzil **4.3d** and **4.3e** were obtained from commercial suppliers. However, the unsymmetrical benzil **4.3a** and the symmetrical benzil **4.3c** were synthesized. Compound **4.3a** was obtained in two steps (Scheme 4.3). Firstly, the corresponding alkyne was synthesized as previously described (*83*), via a Sonogashira coupling from 1-(*tert*-butyl)-4-ethynylbenzene and 1-iodo-4-methoxybenzene, and secondly benzil **4.3a** was obtained in excellent yield via an oxidation of the alkyne, using 2 mol% of PdI_2 and DMSO (*84*). The symmetrical di-*tert*-butylbenzil **4.3c** was synthesized in two steps according to literature (*85*). The corresponding benzoin was synthesized from 4-*tert*-butylbenzaldehyde, and subsequent oxidation of the crude benzoin derivative gave benzil **4.3c** in a total yield of 42%.



Scheme 4.3. Synthetic route and yields of the two benzils that were synthesized. (a) The unsymmetrical benzil **4.3a**. (b) The symmetrical benzil **4.3c**.

The two benzoic acids (**4.4a**-**b**) that were used as reference substances were synthesized as previously described (*86*) and the benzophenones (**4.5a**-**b**) and benzaldehydes (**4.6a**-**b**) were obtained from commercial suppliers.

4.2 Photostability of Dibenzoylmethanes

To simplify the development of a suitable analysis method for the quantification of the photoproducts of **4.1a**, two other symmetrical dibenzoylmethanes, 4,4'-di-*tert*-butyldibenzoylmethane (**4.1c**) and 4,4'-dimethoxydibenzoylmethane (**4.1d**), were first studied (Figure 4.1). Each dibenzoylmethane was dissolved in cyclohexane and in EtOH to study the impact of the solvent polarity on the photodegradation. In cyclohexane, the symmetrical dibenzoylmethanes (**4.1c** and **4.1d**) were illuminated for 60 min, whereas the unsymmetrical dibenzoylmethane **4.1a** required illumination for 90 min to yield amounts large enough for quantification. The photodegradation was considerably slower in EtOH, so therefore the illumination time had to be extended to 90 min for **4.1c** and **4.1d** and to 210 min for **4.1a**.

The outcome of the different photolysis experiments was analyzed by HPLC/MS and GC/MS. Reference compounds were used to identify the obtained photoproducts and standard curves were used for quantification. Analysis of the photodegradation mixtures with our HPLC/MS system showed, more or less, all the photodegradation products that we had expected to find. The obtained UV chromatogram at 265.4 nm from the 90 min photolysis of **4.1a** in cyclohexane can be seen in Figure 4.2.

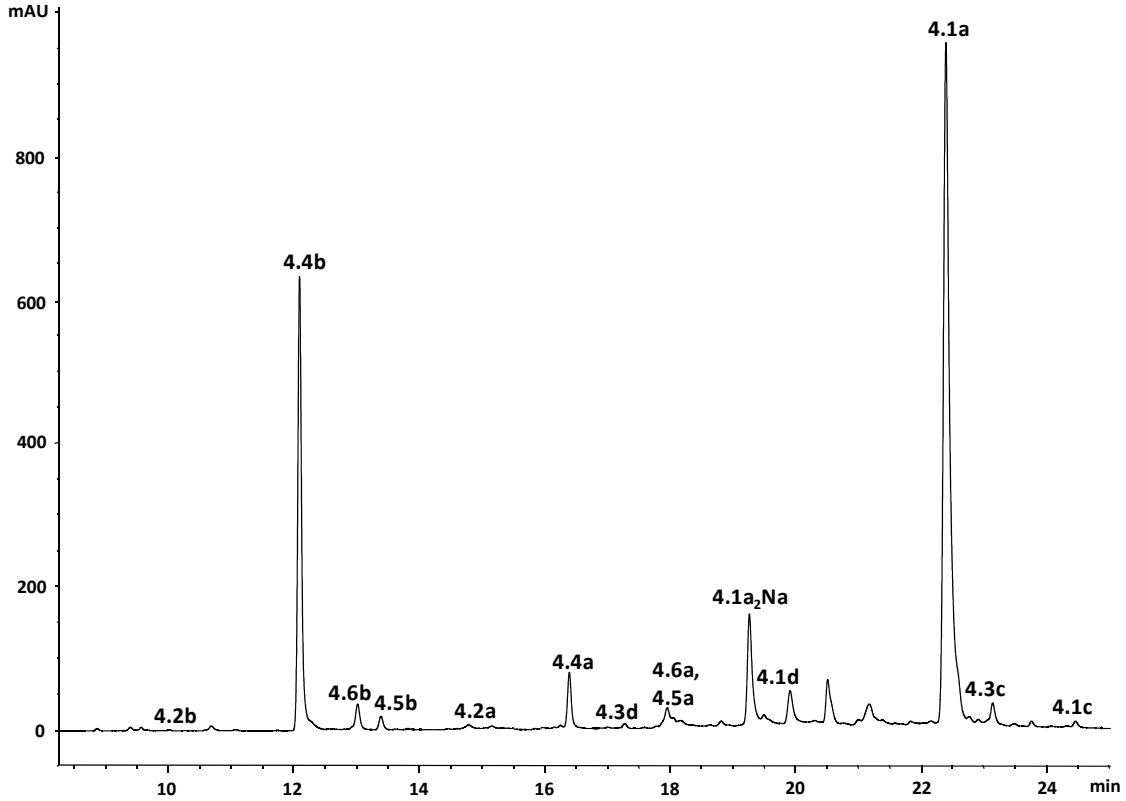


Figure 4.2. HPLC chromatogram at 265.4 nm from analysis of the 90 min photolysis experiment of dibenzoylmethane **1a** in cyclohexane.

In the HPLC/MS system, **4.1a** itself actually gives two different peaks, one at 22.5 min that corresponds to the protonated monomeric adduct, and one smaller peak at 19.2 min that corresponds to the dimeric sodium adduct. Graphs of the formation of the different photodegradation products, identified in the HPLC/MS analysis, in the photolysis experiment with **4.1a** in cyclohexane can be seen in Figure 4.3, and the outcome of the corresponding reaction in EtOH is displayed in Figure 4.4. The results from the photolysis experiments of dibenzoylmethanes **4.1c** and **4.1d** are shown in Appendix I, Figure AI.1 and AI.2.

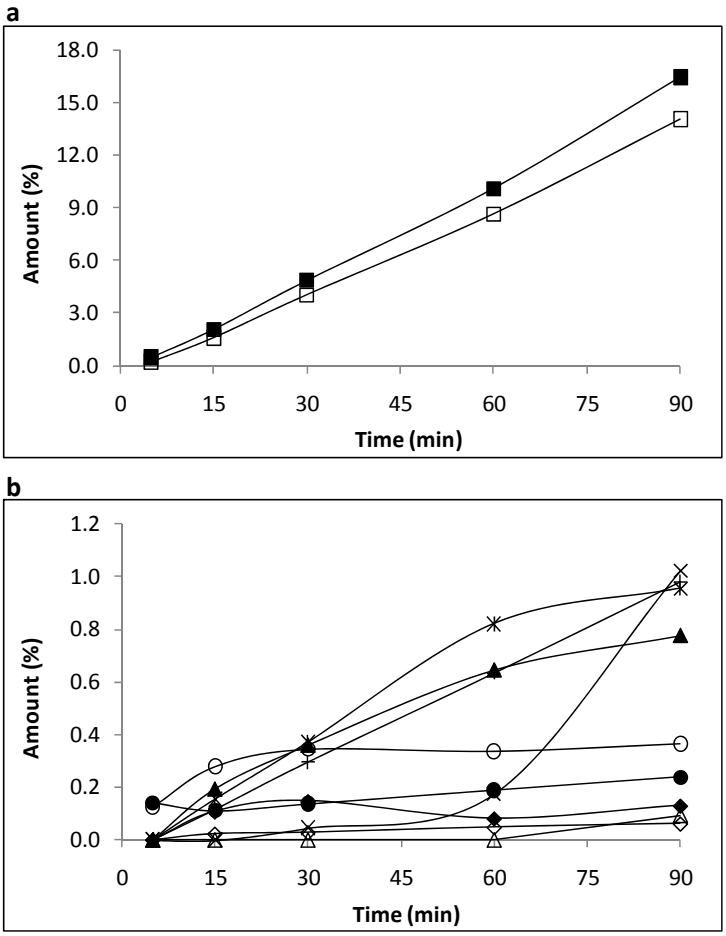


Figure 4.3. Photolysis experiment with dibenzoylmethane **4.1a** in cyclohexane for 90 min. The amount is in relation to the initial concentration of dibenzoylmethane **4.1a**. (a) Formation of benzoic acids **4.4a** (■) and **4.4b** (□). (b) Formation of dibenzoylmethanes: **4.1c** (●) and **4.1d** (o); formation of arylglyoxals: **4.2a** (▲) and **4.2b** (**Δ**); formation of benzils: **4.3c** (♦) and **4.3d** (◊); formation of benzophenones and benzaldehydes: **4.5a** + **4.6a** (ж), **4.5b** (×), and **4.6b** (+).

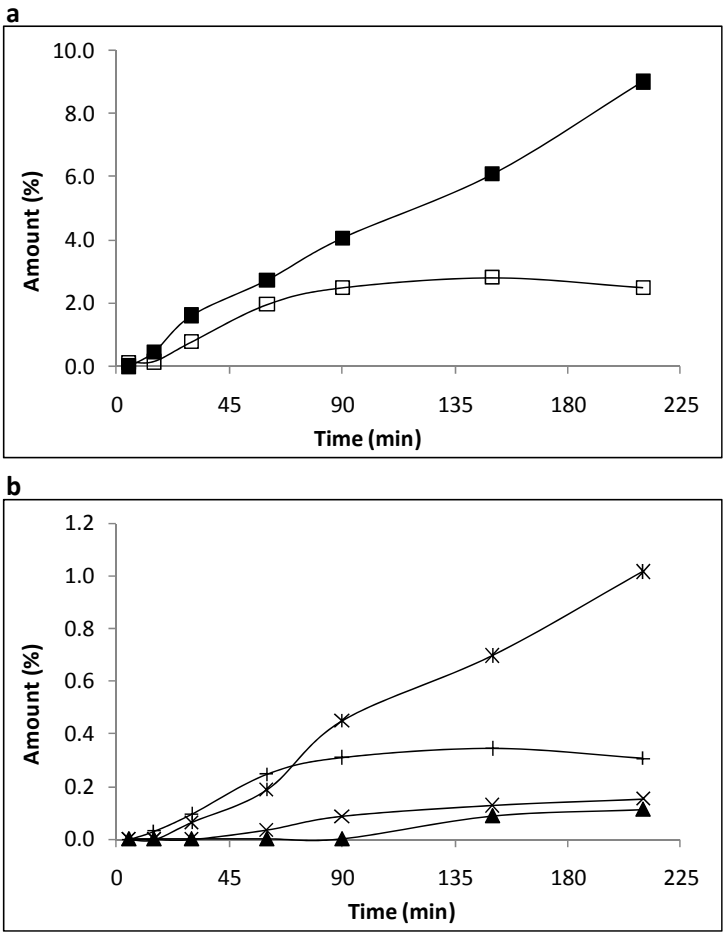


Figure 4.4. Photolysis experiment with dibenzoylmethane **4.1a** in EtOH for 210 min. The amount is in relation to the initial concentration of **4.1a**. (a) Formation of benzoic acids **4.4a** (■) and **4.4b** (□). (b) Formation of arylglyoxal **4.2a** (▲); formation of benzophenones and benzaldehydes: **4.5a** + **4.6a** (ж),**4.5b** (×), and **4.6b** (+).

By far the largest photodegradation products are the two benzoic acids: 4-*tert*-butylbenzoic acid and 4-methoxybenzoic acid, which in cyclohexane constitute approximately 15% each after 90 min photolysis of **4.1a** (Figure 4.3). Other photodegradation products formed in reasonable amounts were benzaldehydes, benzophenones and arylglyoxals. Unfortunately we were not able to develop a method for complete separation of 4-*tert*-butylacetophenone (**4.5a**) and 4-*tert*-butylbenzaldehyde (**4.6a**) (Figure 4.2), so these are quantified together. A compilation of the concentrations of the different photodegradation products formed from **4.1a** after 90 min photolysis in cyclohexane are given in Table 4.1.

Table 4.1. Product concentrations after 90 minutes irradiation of dibenzoylmethane 4.1a

photodegradation product	concentration (mM)	% of initial concentration of **4.1a**
4.4a	0.3287	16.43
4.4b	0.2812	14.06
4.5a + **4.6a**	0.0191	0.95
4.5b	0.0204	1.02
4.6b	0.0196	0.98
4.1c	0.0048	0.24
4.1d	0.0073	0.37
4.2a	0.0155	0.78
4.2b	0.0018	0.09
4.3c	0.0026	0.13
4.3d	0.0012	0.06

Arylglyoxals were detected in all photolysis experiments and after 90 min irradiation of **4.1a**, **4.2a** constitute 0.78% and **4.2b** 0.09% (Table 4.1). In the corresponding experiment in EtOH only **4.2a** could be detected and after 210 min of photolysis it corresponds to approximately 0.1%. Benzils could be detected in all photodegradation experiments performed in cyclohexane, but not in any of the EtOH experiments. In the photolysis experiments of **4.1a** in cyclohexane all three benzils that can be formed (**4.3a**, **4.3c-d**) was identified in the mixture and the two symmetrical ones (**4.3c**, **d**) were also quantified. Unfortunately, the unsymmetrical benzil (**4.3a**) coeluated with other compounds and could therefore not be quantified. In the photolysis experiment with **4.1a**, the two symmetrical dibenzoylmethanes (**4.1c** and **4.1d**) could be detected when cyclohexane was used as solvent, but not when the experiment was conducted in EtOH. Both the benzils and the symmetrical dibenzoylmethanes are recombination products, so it therefore seems as if the formation of these kinds of adducts are favoured by a non-polar solvent like cyclohexane.

In contrast to the HPLC/MS analysis, the GC/MS analysis of all three photodegradation experiments in cyclohexane showed large amounts of the oxidized cyclohexane products **4.9** and **4.10** as well as the cyclohexane adducts **4.7a**-**b** and **4.8a**-**b**. Note that no reference compounds were used for the identification of compounds **4.7a**-**b**, **4.8a**-**b**, **4.9** and **4.10**; the structures of these compounds were based on their masses, obtained in the GC-MS analysis, and the fact that these compounds were identified in a similar study done by Roscher et al. (*46*). Some of the photodegradation products detected with the HPLC/MS system were also detected with the GC/MS system. However, it was not possible to identify or quantify many of the photodegradation products formed in smaller amounts with our GC/MS system, due to interfering peaks from solvent adducts and solvent oxidation products. A representative chromatogram from the GC/MS analysis of the 60 min photolysis of dibenzoylmethane **4.1d** can be seen in Figure 4.5.

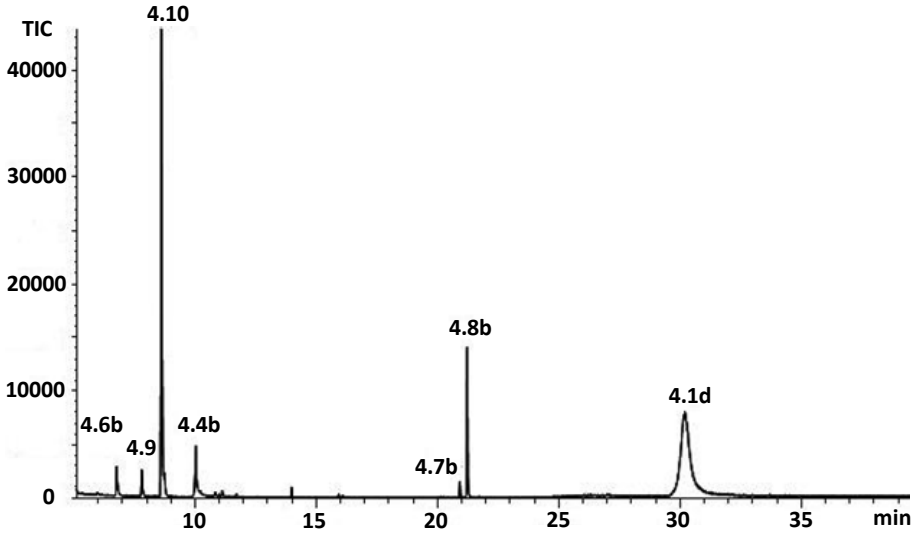
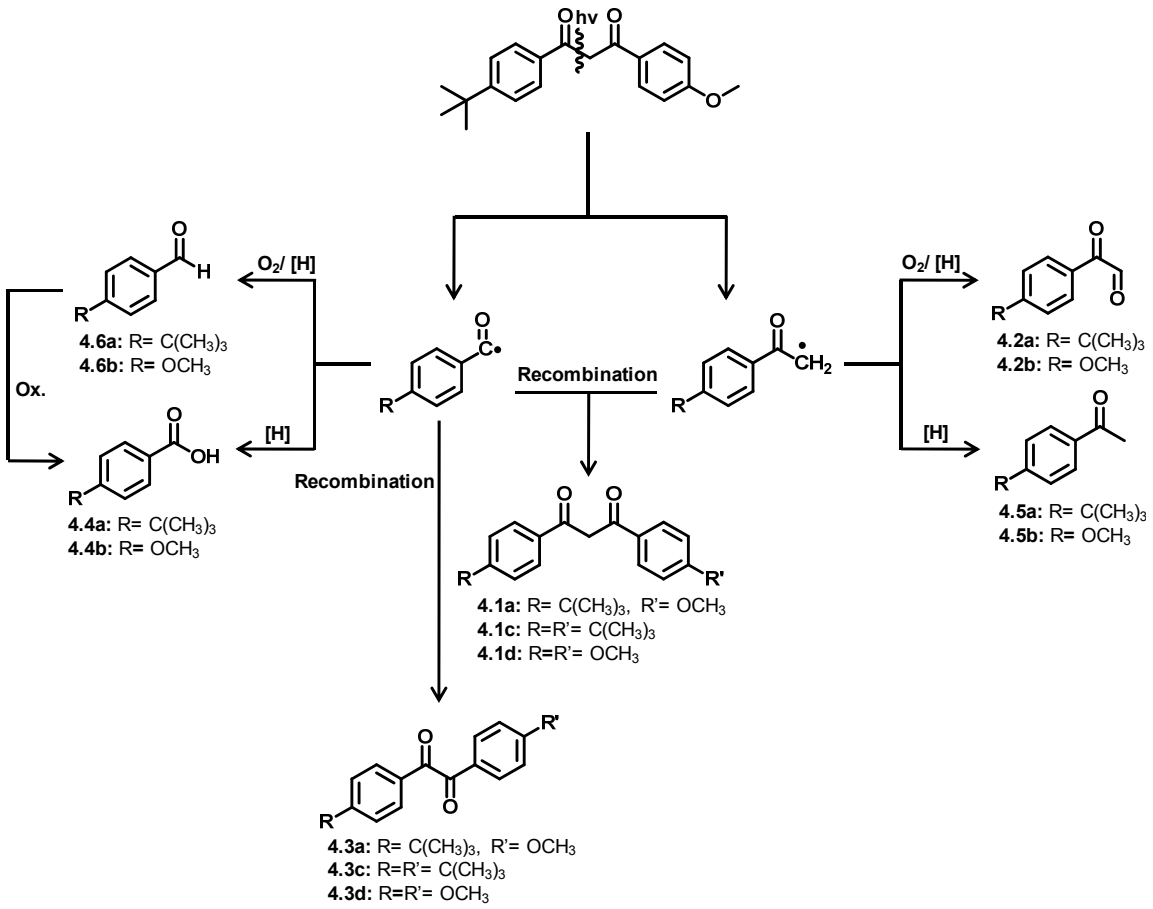


Figure 4.5. GC/MS chromatogram from the 60 min photolysis of dibenzoylmethane **4.1d**.

Our results from the photodegradation experiments of dibenzoylmethanes **4.1a-d** are in good agreement with those reported in the literature (*45, 46*). The extensive formation of cyclohexane oxidation products (compound **4.9** and **4.10**) and cyclohexane adducts (compound **4.7a**-**b** and **4.8a**-**b**), which we observed when we analyzed the photolysis mixtures with our GC/MS system, has previously been observed by Roscher et al. (*46*), whereas the observed photodegradation adducts detected with our HPLC/MS system are in agreement with the results obtained by Schwack and Rudolph (*45*). In the study by Schwack and Rudolph, no quantification was performed; only a qualitative analysis was done, so therefore it is not possible to do a quantitative comparison. However, the detected compounds were the same with the exception of the dibenzoylmethanes (Scheme 1.1, section 1.5.1), which were not observed in any of our experiments. Scheme 4.4 shows a tentative mechanism for the photodegradation of **4.1a**. Another discrepancy between the results from our study, compared to the previous one, is the degradation of **4.1a** in EtOH. Even if the degradation of all three dibenzoylmethanes were considerably slower in EtOH than in cyclohexane, we did see a clear degradation, which Schwack and Rudolph (*45*) did not. This difference is most likely due to differences in the reaction conditions, such as oxygen supply, irradiation time and irradiation source. In our study, air was continuously bubbled through the samples to avoid oxygen depletion, and the irradiance of the light source used in our experiments was higher. Also, somewhat shorter wavelengths were used ($\lambda > 240$ nm compared to ($\lambda > 260$ nm).



Scheme 4.4. Proposed photodegradation pathway for dibenzoylmethane **4.1a**. [H] stands for H-abstraction and in this experiment the hydrogen is most likely abstracted from the solvent. Ox. stands for oxidation.

4.3 Assessment of Sensitizing Potency

Of the photodegradation products formed from the dibenzoylmethanes in the photolysis experiments, the sensitizing potency of the benzoic acids (*87*), benzophenones (*88*) and benzaldehydes (*89*) had previously been studied and they were all shown to be nonsensitizers. However, to the best of our knowledge, the sensitizing potency of arylglyoxals and benzils has not previously been studied. Since many haptens have electrophilic properties, we anticipated that especially the arylglyoxals would posses sensitizing characteristics. In addition, both glyoxal (EC3 = 0.74%) and methylglyoxal (EC3 = 0.42%) have been shown to be strong sensitizers in the LLNA (Structures can be seen in Figure 4.6) (*90*).

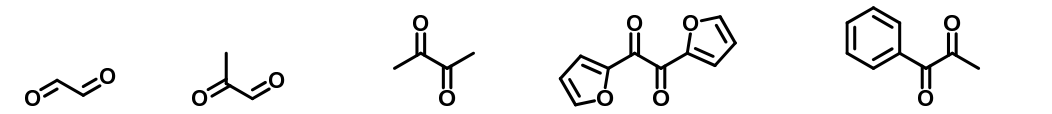


Figure 4.6. Structures and EC3-value of glyoxals and diketones previously assessed in the LLNA.

In total four different arylglyoxals (**4.2a**-**d**) were chosen for assessment of their sensitizing capacity in the LLNA. Arylglyoxals **4.2a** and **4.2b** are formed in photodegradation of dibenzoylmethane **4.1a**, and **4.2c** is formed in photodegradation of dibenzoylmethane **4.1b**. Arylglyoxal **4.2b** has a strong electron-donating *para* substituent, whereas both **4.2a** and **4.2c** have weak electron-donating *para* substituents. Therefore, in order to fully explore what influence different *para* substituents has on the sensitizing potency of arylglyoxals, a fourth synthetic analog with a strong electron-withdrawing *para* substituent was included in the test series.

The benzils are also electrophilic but not as electrophilic as the arylglyoxals, so it was not as easy to predict their sensitizing ability. Furthermore, LLNA studies of other diketones such as 2,3-butadione, furil, and 1-phenyl-1,2-propanedione have resulted in very varying results, ranging from classification of 1-phenyl-1,2-propanedione (EC3 = 1.3%) as potent sensitizer to furil as a nonsensitizer (*91*); see Figure 4.6 for structures of the diketones.

The results from the murine LLNA, used to determine the sensitizing capacity of two dibenzoylmethanes (**4.1a** and **4.1b**), four arylglyoxals (**4.2a**-**d**), and four benzils (**4.3a** and **4.3c**-**e**), are shown in Table 4.2 and Figure 4.7. Tables with the values for dpm/lymph node and SI for each test concentration can be found in Appendix, Table AI.1 (dibenzoylmethanes and arylglyoxals) and Table AI.2 (benzils). Compound **4.1b**, the dibenzoylmethane used in sunscreens in the eighties and early nineties, turned out to be a weak sensitizer with an EC3 of 1.2 M or 32.5%, and the dibenzoylmethane used in sunscreens today (**4.1a**) was classified as a nonsensitizer (Table 4.2). On the other hand, the arylglyoxals (**4.2a**-**d**), were all shown to be very strong sensitizers with EC3 values ranging from 0.0092 to 0.016 M or 0.16 to 0.32% (Table 4.2). It is noteworthy that the LLNA results, within experimental variations, were the same for all arylglyoxals despite the difference in electronic effects of their para substituents.

Table 4.2. EC3 values and classifications obtained from the LLNA experiments

test compound	EC3 value		classification
	% (w/v)	M	
4.1a	----	----	nonsensitizing
4.1b	32.5	1.2	weak
4.2a	0.22	0.011	strong
4.2b	0.16	0.0092	strong
4.2c	0.32	0.016	strong
4.2d	0.23	0.012	strong

Two of the benzils (**4.3a** and **4.3d**) were classified as nonsensitizers. However, due to low solubility in the vehicle, benzil **4.3d** was only tested up to 2%. Unexpectedly, the other two benzils (**4.3e** and **4.3c**) gave curves that did not correspond to a dose-response behavior (Figure 4.7). One explanation for this different result could be that the compounds are toxic.

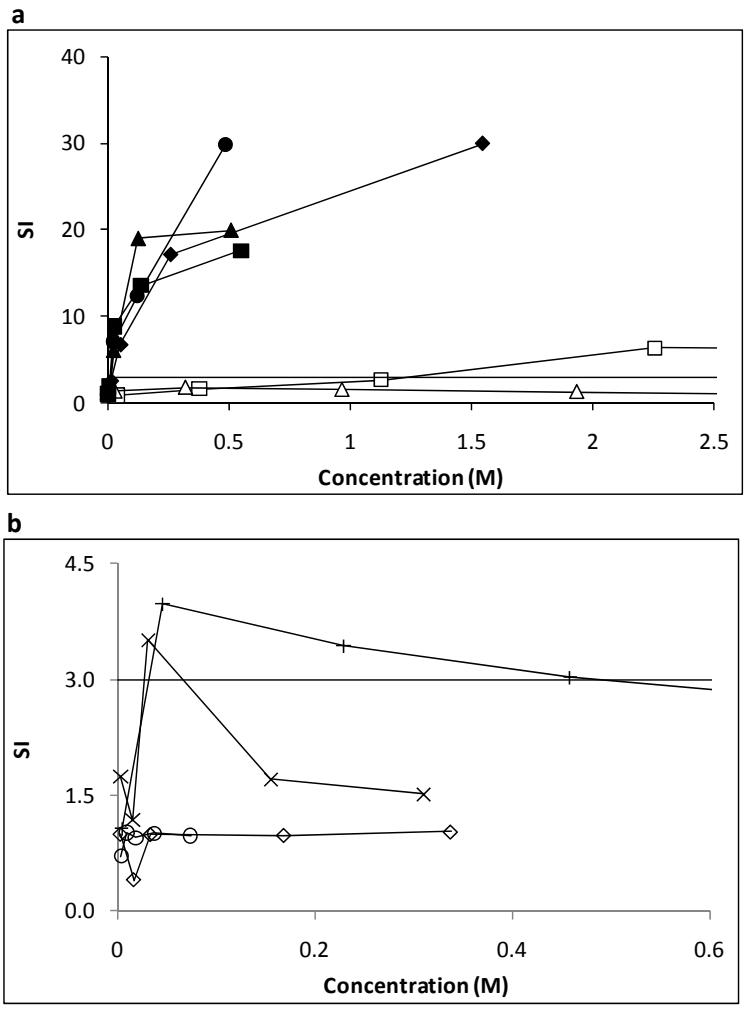


Figure 4.7. Dose-response curves for the substances assessed in the LLNA. The concentrations are given in molar. The horizontal line marks an SI of 3, the cutoff limit for a compound to be considered a sensitizer. (a) Dibenzoylmethanes **4.1a** (Δ) and **4.1b** (□); arylglyoxals **4.2a** (●),**4.2b** (■),**4.2c** (♦) and **4.2d** (▲). (b) Benzils **4.3a** (◊),**4.3c** (×), **4.3d** (○), and **4.3e** (+).

4.4 Cell Toxicity of Benzils

To find out whether the deviation from a dose response in the LLNA for **4.3c** and **4.3e** was due toxicity, an MTS cell viability assay was conducted on all four benzils. Other benzils have previously been shown to posses both cytotoxic and antiproliferative activity (*92, 93*). Therefore, toxicity seemed like a probable explanation for the observed LLNA responses for **4.3c** and **4.3e**. Indeed, all benzils were cytotoxic in the two lower test concentrations, 0.125 and 0.25 mM (Figure 4.8).

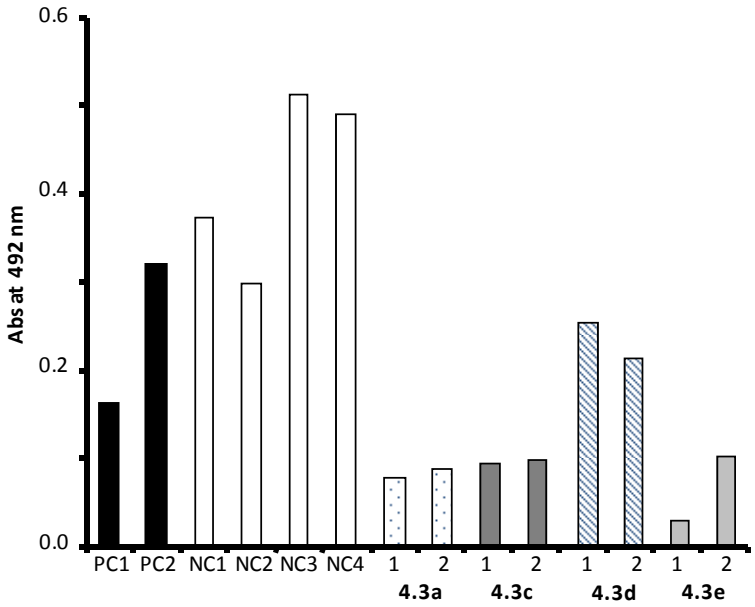


Figure 4.8. The cytotoxicity of the benzils was assessed with an MTS cell viability assay. The shown results are the mean absorbance of triplicates for each benzil or control at 492 nm after 24 h incubation followed by 2 h incubation after the addition of MTS/PMS. Positive controls: PC1 0.25 mM $CuSO_4$ and A2 0.125 mM $CuSO_4$. Negative controls: NC1 medium only; NC2 medium with 1.0% EtOH; NC3 medium with 2.0% EtOH; NC4 medium with 1.8% EtOH and 0.20% DMSO; Benzils: **4.3a**1 0.25 mM and **4.3a**2 0.125 mM; **4.3c**1 0.25 mM and **4.3c**2 0.125 mM; **4.3d**1 0.25 mM and **4.3d**2 0.125 mM; **4.3e**1 0.25 mM and **4.3e2** 0.125 mM.

At the highest concentrations tested (1.0 mM for **4.3a**, **4.3c**, **4.3e** and 0.5 mM for **4.3d**) all benzils formed crystals or oil droplets in the water-based cell medium (Figure 4.9); therefore, these results were excluded. Unfortunately, benzil **4.3d** formed crystals at all test concentrations. Hence, the higher absorbance obtained for benzil **4.3d** (Figure 4.8) is probably not due to a lower toxicity as such, but is instead caused by the light scattering caused by the crystals during the UV-vis measurement, in combination with the lower concentration of **4.3d** in solution. The fact that the lowest concentration of **4.3d** gives the lowest absorbance further supports this reasoning. Because of this poor solubility of **4.3d** it was not possible to accurately determine its cytotoxicity by this method. However, visual examination with a light microscope, after staining with tryphan blue, clearly showed that also this benzil had a toxic effect on the cells. Figure 4.9 shows images of healthy cells from the negative controls and of cells treated with benzils, showing cells that are deformed and fragmented.

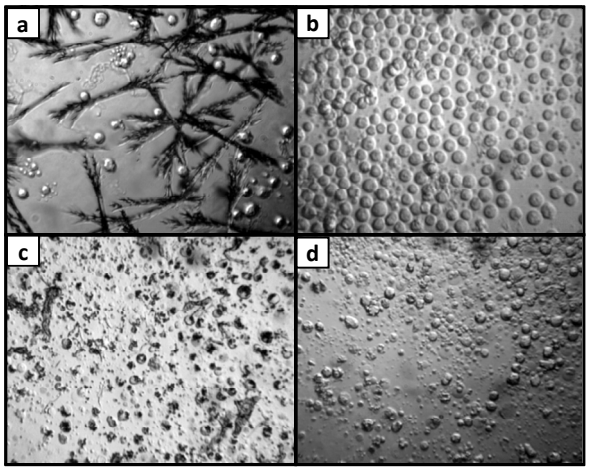


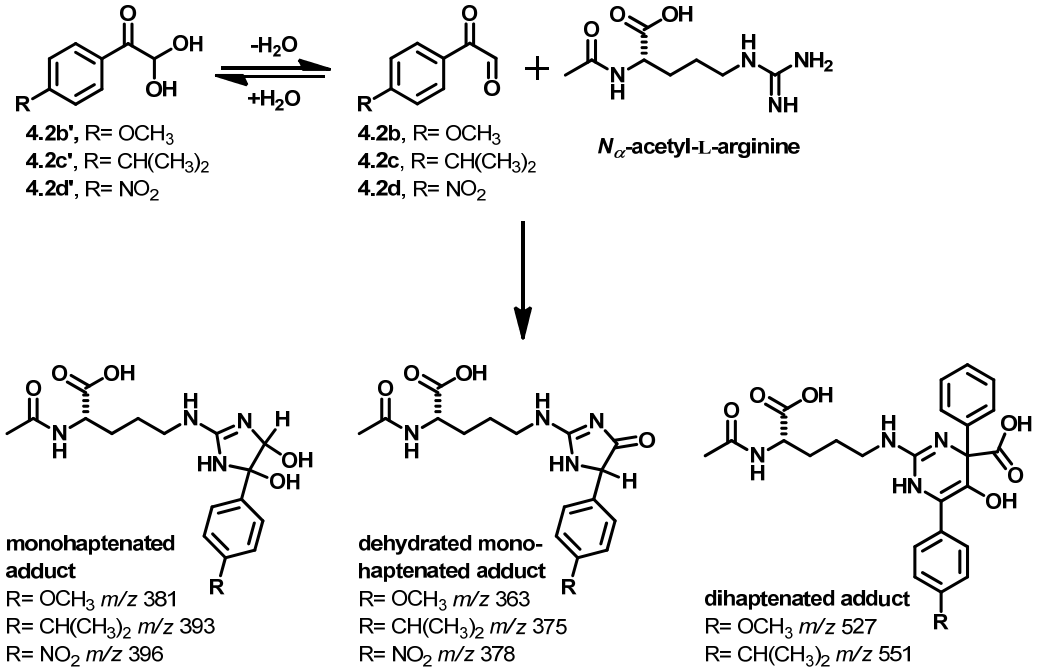
Figure 4.9. Visual examination of cells used in the cytotoxicity experiment with benzils. (a) Crystals formed at 1 mM concentration of benzil **4.3e** in medium. (b) Cells incubated with dilution medium (RPMI-1640 with 1% EtOH) only, with a healthy characteristic round shape. (c) Deformed cells after incubation with 0.25 mM of benzil **4.3a**. (d) Deformed cells after incubation with 0.25 mM of benzil **4.3e**.

According to the LLNA results only two of the benzils (**4.3c** and **4.3e**) seem to be toxic, but the MTS assay gives approximately the same result for at least three of the benzils (**4.3a**, **4.3c** and **4.3e**). One hypothesis is that the observed difference between these two tests may have to do with the compounds solubility. Both 4-*tert*-butyl-4'-methoxybenzil **4.3a** and especially 4,4'-dimethoxybenzil **4.3d** are less soluble than the other two. In the cell viability assay the compounds are added directly to the cells, whereas they in the LLNA are added to the outside of the mouse ear. Therefore, high enough concentrations of the less soluble benzils may not penetrate deep enough to cause a toxic or allergic response in the mouse.

4.5 Chemical Reactivity of Arylglyoxals

All four arylglyoxals tested in the LLNA were demonstrated to be very potent sensitizer and although they have *para* substituents with varying electronic properties there were no real differences in their sensitizing capacities (Table 4.2). Most haptens form immunogenic hapten-protein complexes via an electrophilic-nucleophilic reaction between the hapten and a protein. Therefore, we had expected to see a difference in sensitizing capacity between the four arylglyoxals. Arylglyoxal **4.2d** would be predicted to be the most sensitizing compound since it has an electron-withdrawing substituent, which ought to make it more electrophilic than the other three arylglyoxals with electron-donating *para* substituents. A chemical reactivity experiment was performed to determine whether this lack of difference in sensitizing capacity is due to similar reactivity or other aspects such as penetration. Both **4.2a** and **4.2c** have a weak electron-withdrawing substituent, so therefore only **4.2c** was included in the reactivity experiment together with **4.2b** and **4.2d**. Although the amino acids cysteine and lysine are most often considered in the formation of immunogenic hapten-protein complexes, in this study we chose the protected amino acid arginine as a model for skin proteins. The reason for this is that phenylglyoxal has been shown to be specific for this amino acid (*94*). The reactions were performed in 20% DMSO in phosphate buffer pH 7 at room temperature. This amount of DMSO had to be added to dissolve the arylglyoxals and the amino acid. The reaction rates were measured as the depletion of each arylglyoxal. The arylglyoxals and their corresponding hydrates are in rapid equilibrium at the conditions used

during these experiments, which prevents the two forms from separating in the HPLC. Therefore, a measure of the total concentration of glyoxal and hydrate can be performed in the HPLC analysis. Moreover, at the reaction conditions used the equilibrium is largely shifted to the hydrate form. However, a small portion of reactive glyoxal is always present because of the rapid equilibrium (Scheme 4.5).



Scheme 4.5. Reaction scheme for the chemical reactivity experiment with arylglyoxals **4.2b-d** and the protected amino acid arginine. Probable structures for the detected adducts are also shown (*95*).

All three arylglyoxals reacted with N_{α}-acetyl-L-arginine at approximately the same rate (Figure 4.10). In all three reactivity experiments, four adducts with *m/z* corresponding to monohaptenated adducts were detected, i.e. *m/z* of 381, 393 and 396 for **4.2b**, **4.2c** and **4.2d**, respectively. The MS-chromatograms and mass spectra for each reaction system can be found in Appendix I. The formation of four different adducts with the same mass is consistent with the fact that the monohaptenated adduct can give four different diastereoisomers (Scheme 4.5). In all three systems, one or two adducts with *m/z* corresponding to monoconjugates of arylglyoxal and arginine minus water were also observed, which also agrees with the fact that two different isomers are possible for the dehydrated monohaptenated adduct (Scheme 4.5). Finally, in the reactivity experiments with **4.2b** and **4.2c**, two adducts with *m/z* corresponding to a dihaptenated adduct could also be seen; see Appendix I. The proposed structures in Scheme 4.5 have been adopted from the work by Saraiva et al. (*95*). Furthermore, in all three reactivity experiments, signals with *m/z* corresponding to monohaptenated adducts minus two, minus 19 and minus 20 were also observed (Appendix I); however, these structures are unknown. Overall, the results from these reactivity experiments agree well with previous studies of phenylglyoxals reactivity toward arginine (*94, 95*).

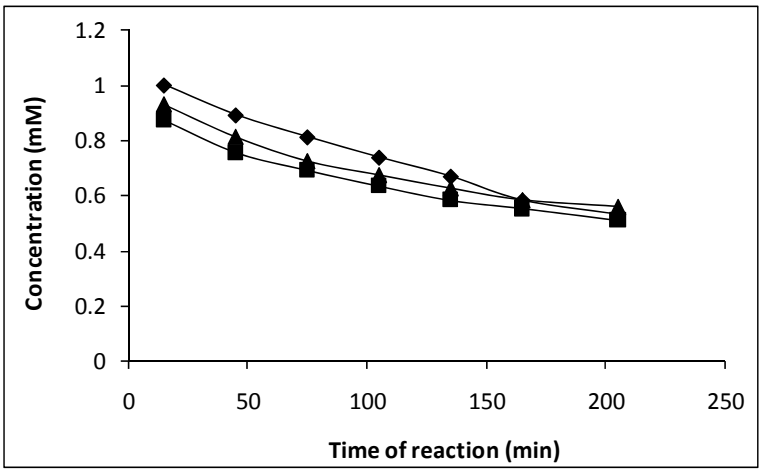


Figure 4.10. Depletion of the arylglyoxals **4.2b** (■), **4.2c** (♦) and **4.2d** (▲) upon reaction with N_{α}-acetyl-L-arginine.

The experiment showed that there was no difference in reactivity of the different arylglyoxals toward the amino acid arginine (Figure 4.10), which is in accordance with the similar results obtained for all arylglyoxals in the LLNA (Table 4.2). One possible explanation for this observation could be that for a more reactive arylglyoxal the equilibrium is shifted more toward the hydrate, which is unreactive toward the nucleophile arginine. In other words, in aqueous media the higher reactivity is moderated by a lower available concentration of the reactive glyoxal form.

4.6 Concluding Discussion

4-*tert*-Butyl-4'-methoxy dibenzoylmethane (**4.1a**) is a UVA-filter that is commonly used in both sunscreens and other skin products, but it is also one of the most common photoallergens. In this study we have shown that dibenzoylmethane **4.1a** photodegrades producing potentially health hazardous compounds, such as benzils and arylglyoxals are formed. The benzils formed from **4.1a** were shown to be cytotoxic rather than allergenic, whereas the arylglyoxals were classified as strong skin sensitizers in the murine LLNA. Although, the exact mechanism for the formation of an immunogenic complex in PACD is not fully understood, our results suggests that photocontact allergy to dibenzoylmethanes is caused by the arylglyoxals that, via an electrophilic-nucleophilc pathway, forms an immunogenic hapten-protein complex. It has been shown that upon repeated application, even very low doses of haptens can induce sensitization (*96, 97*). Since a sun lotion is often applied repeatedly, even the small amounts of arylglyoxals that are formed, when dibenzoylmethanes photodegrade, may be enough to cause sensitization. Furthermore, we have shown that the arylglyoxals *para* substituent does not influence either their sensitization potency, or their chemical reactivity toward the nucleophile arginine. Based on these findings, it is probably not possible to lower the photoallergenic potency of dibenzoylmethanes by simply changing their *para* substituents, which seems to have been the industries way of dealing with this adverse side effect, at least in the past.

Another possible route for the formation of an immunogenic complex, from illuminated dibenzoylmethanes, is via a reaction of the benzoyl radical with a protein. This mechanism is supported by the formation of the compounds **4.7a**-**b** and **4.8a**-**b**. This was not explored further. However, it would have been highly interesting to do photochemical reactivity experiments of dibenzoylmethane **4.1a** toward different amino acid analogs to find out which of these two mechanisms would dominate. This could also be investigated in patients with known photocontact allergy to dibenzoylmethanes. A positive patch test to any of the corresponding arylglyoxals would suggest that they are responsible for the photoallergenic potency of the dibenzoylmethanes, whereas a negative patch test would imply that it is the benzoyl radical that causes the sensitization. If some patients display positive patch test results and some negative, it would suggest that different patients are sensitized to different immunogenic complexes and that both routes are of clinical relevance.

5 STUDIES OF OCTOCRYLENE (PAPER II AND III)

Octocrylene is a relatively new commercial UV-filter. It was introduced in the nineties, but it is not until this last decade that the use of it has really increased. For this reason, the number of allergic reactions has not been a problem until recently. In an Italian multicenter study from 2004 to 2006, 23 of the 1082 patients that were patch and photopatch tested displayed a positive test reaction to octocrylene (*38*). The only tested allergen that gave more positive reactions in that study was ketoprofen. Last year (2010), clinics in France and Belgium, gathered under REVIDAL (*98*), reported as many as 50 positive patch and photopatch reactions to octocrylene after testing of patients with adverse skin reactions to sunscreens or ketoprofen (*53*). Interestingly, all children but one displayed a positive patch test, whereas most adults only displayed a positive result in the photopatch test. Further, many of the patients in the REVIDAL study with positive photopatch tests to octocrylene had a history of photocontact allergy from ketoprofen.

The aim of this study was to investigate octocrylene's chemical (Paper II) and photochemical (Paper III) properties, in order to explain the relatively high number of allergic reactions that are reported. We also initiated collaboration with a clinic in Belgium to further study the frequency of allergic reaction caused by octocrylene, as well as the occurrence of contemporary reactions to octocrylene, ketoprofen and benzophenone-3 (Paper II); structures of the compounds can be seen in Figure 5.1.

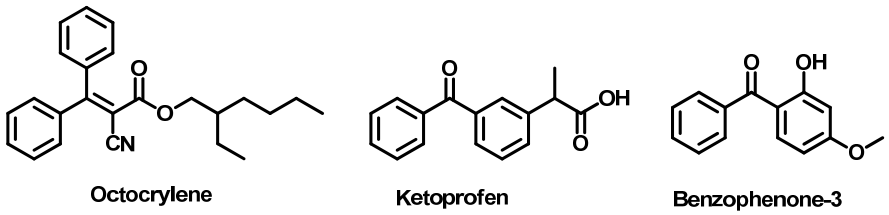


Figure 5.1. Structures of the compounds discussed in this study.

5.1 Clinical Studies (Paper II)

In total 172 patients with adverse skin reactions to sunscreens and/or ketoprofen were patch and photopatch tested and 23 of these displayed a positive test reaction to octocrylene. Of the octocrylene positive patients, 5 reacted already in the patch test, whereas the other 18 only reacted to octocrylene in the photopatch test (Table 5.1).

Table 5.1. Results from patch- and photopatch testing for the 23 octocrylene positive patients.

Case	Age/ Gender	Sunscreen intolerance*	Use of topical ketoprofen*	octocrylene		ketoprofen		benzophenone-3	
				PT	PPT	PT	PPT	PT	PPT
1	41/F	YES	YES	-	++	-	+++	-	++
2	71/M	YES	YES	-	+	-	++	-	-
3	56/F	YES	?	+	+	NT	NT	-	-
4	44/M	YES	?	-	+	NT	NT	-	-
5	41/F	YES	YES	-	++	-	++	-	+
6	48/F	YES	NO	-	++	NT	NT	-	-
7	38/M	YES	YES	-	++	++	+++	-	+
8	7/F	YES	NO	-	+	NT	NT	-	-
9	41/F	NO	YES	-	+	+	++	-	+
10	3/F	YES	NO	+	+	-	-	-	-
11	11/F	YES	NO	+	NT	NT	NT	-	-
12	43/F	YES	YES	-	++	-	+++	-	+
13	29/F	YES	YES	-	+	-	++	-	-
14	33/M	YES	YES	+	+++	-	+++	-	++
15	54/M	NO	YES	-	+	-	++	-	+
16	31/M	YES	YES	-	+++	-	+++	-	++
17	36/F	YES	YES	+	+	-	+++	-	+++
18	53/F	YES	YES	-	+++	-	+++	-	+
19	42/F	YES	YES	-	++	-	+++	-	++
20	33/F	NO	YES	-	++	-	+++	-	++
21	24/M	YES	YES	-	++	-	++	-	+
22	24/M	YES	YES	-	+	-	+++	-	+
23	32/F	YES	YES	-	++	-	++	-	++

PT: patch test, PPT: photopatch test, NT: not tested

* According to the patient's own statement.

Reactions are graded according to the International Contact Dermatitis Research Group recommendations (*67*): +++ = vesicles, papules, erythema and infiltrate; ++ = papules, erythema and infiltrate; + = erythema and infiltrate.

Of the five patients that reacted positive to octocrylene in the patch test, one had an augmented reaction to octocrylene in the photopatch test, one were not photopatch tested with octocrylene and the remaining three presented with the same degree of reaction in both the patch and the photopatch test. According to the patient's own statements, 20 of the 23 octocrylene positive patients had experienced an adverse reaction to a sunscreen and 17 had a history of topical ketoprofen use. All of the 17 patients that had used topical ketoprofen were tested with it and they all reacted positive on the photopatch test. Five of the six patients that had never used topical ketoprofen were unfortunately not tested with it. However, the one patient that was tested with ketoprofen did not react to it. On the other hand, all 23 octocrylene positive patients were tested with benzophenone-3 and interestingly 15 of the 17 patients that reacted positively to ketoprofen also displayed a positive photopatch reaction to benzophenone-3, whereas neither of the patients that had

not used ketoprofen reacted to benzophenone-3. This high concordance of positive photopatch reactions to both octocrylene and ketoprofen, seen in this study, is in agreement with earlier reports (*53, 56*).

According to both our study as well as previous ones, it appears as if ketoprofen leads to photocontact allergy to octocrylene and in the majority of cases also to benzophenone-3. All octocrylene positive patients were not tested with ketoprofen, so unfortunately it is not known whether the opposite applies, i.e. if octocrylene leads to photocontact allergy to ketoprofen. However, none of the patients that had never used topical ketoprofen reacted positively to benzophenone-3. This indicates that sensitization to octocrylene does not lead to photocontact allergy to either ketoprofen or benzophenone-3. Although, most of the patients in this study only displayed a positive photopatch test reaction to octocrylene, approximately 20% reacted already in the patch test, which implies that octocrylene causes both contact and photocontact allergy. Other clinical studies have also reported both positive patch and photopatch test reactions to octocrylene (*38, 52, 53, 55*). In these studies the incidence of patients reacting already in the patch test was 35-40%, which is in fact even higher than in our study. As mentioned previously, it was pointed out in the study made by Avenel-Audran et al. (*53*) that it is mostly children that display positive patch tests to octocrylene. Also, in this study 2 of the 3 octocrylene positive children reacted already in the patch test.

5.2 Sensitizing Potency of Octocrylene (Paper II)

The result from the evaluation of octocrylene's sensitizing capacity in the murine LLNA can be seen in Table 5.2 and Figure 5.2. It was classified as a moderate sensitizer with an EC3 value of 0.21 M or 7.7%. The approved maximum concentration of octocrylene, by both the EU and the FDA, is 10%. Therefore, it is not surprising that this moderate allergen can induce sensitization in patients, even without any activation by UV radiation.

Table 5.2. [^{3}H]-thymidine incorporation (dpm/lymph node) and SI values for octocrylene tested in the LLNA.

Concentration	dpm/ lymph node	SI
Control	251	1.00
1.0% (0.028 M)	327	1.30
2.5% (0.069 M)	410	1.63
5.0% (0.14 M)	563	2.25
15% (0.42 M)	1260	5.01
30% (0.83 M)	1100	4.38
EC3: 7.7% (0.21 M)		

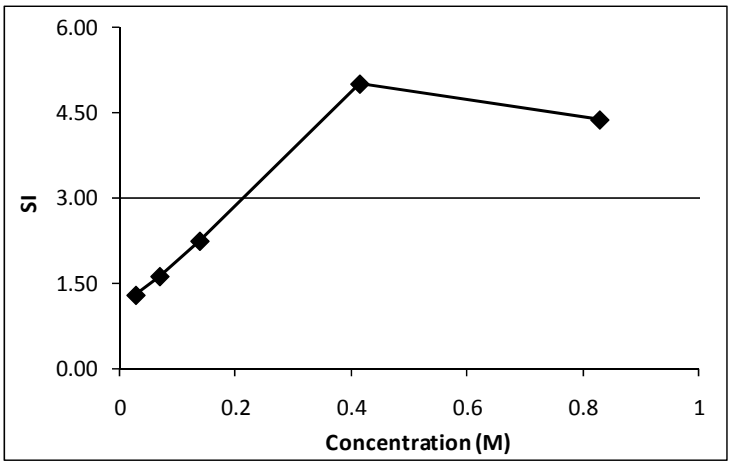


Figure 5.2. Dose-response curve for octocrylene tested in the LLNA. The concentrations are given in molar. The horizontal line marks an SI of 3, the cutoff limit for a compound to be considered a sensitizer.

5.3 Chemical Reactivity of Octocrylene (Paper II)

Octocrylene was shown to be a moderate sensitizer in LLNA, so to investigate whether octocrylene itself is reactive, or if metabolic activation is required to transform it into a hapten, chemical reactivity experiments were performed. Octocrylene is an α,β-unsaturated carbonyl compound and this class of substances are considered to be specific for the amino acid cysteine (*99-102*), i.e. sulfur nucleophiles. Therefore, the reactivity of octocrylene toward N_{α}-acetyl-L-cysteine-methyl ester was first studied. However, no depletion of octocrylene or formation of any adducts could be detected with our HPLC/MS method, even after 9 days. On the other hand, when the lysine analog benzylamine was used as an amino nucleophile, approximately 60% of octocrylene had reacted after 8 hours (Figure 5.3). To further investigate if octocrylene displayed the same reactivity toward the amino acid lysine itself, a reactivity experiment of octocrylene toward N_{α}-acetyl-L-lysine-methyl ester was also performed. The protected amino acid lysine was not soluble in EtOH, the solvent that was used in the two first reactivity experiment, so the alternative solvent system ACN/carbonate buffer pH 10.0 (3:1) was used instead. A fourth reactivity experiment between octocrylene and benzylamine in ACN/carbonate buffer pH 10.0 (3:1) was also carried out to investigate if the different solvent systems had any effect on the reaction rate. It was shown that there was no difference in reaction rate between the two amines (benzylamine and N_{α}-acetyl-L-lysine-methyl ester), see Figure 5.3. When the same solvent system was used (ACN/carbonate buffer pH 10.0 (3:1)) and pseudo-first-order kinetics was assumed, the exact same rate constant (k = 0.055 hr^{-1}) was obtained for these two nucleophiles (Figure 5.3). The solvent, on the other hand, had a clear effect on the reaction rate. The obtained rate constant was twice as high when EtOH was used as solvent (k = 0.109 hr^{-1}) compared to ACN/carbonate buffer pH 10.0 (3:1) (k = 0.055 hr^{-1}) (Figure 5.3).

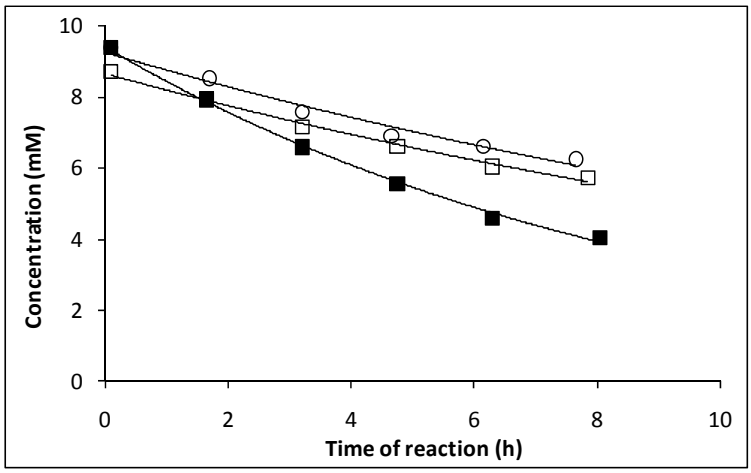
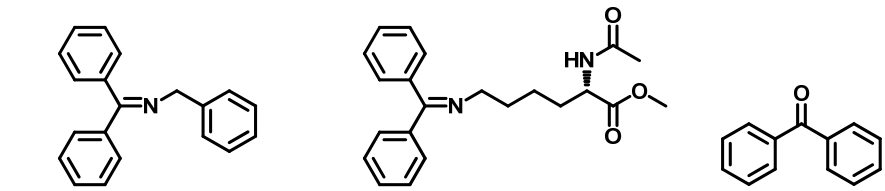


Figure 5.3. Depletion of octocrylene using two different nucleophiles and two different solvent systems: (○) k = 0.055 hr^{-1} for N_α-acetyl-L-lysine methyl ester in ACN/carbonate buffer pH 10.0 (3:1), (□) k = 0.055 hr^{-1} for benzylamine in ACN/carbonate buffer pH 10.0 (3:1), and k = 0.109 hr^{-1} for (■) benzylamine in EtOH. The initial concentrations were 10 mM of octocrylene and 100 mM of nucleophile. The lines were obtained by applying an exponential fitting to the data points. The slopes k corresponds to the pseudo-first order rate constants for the reactions.

The different products formed in the reactivity experiments with octocrylene were shown to be the corresponding benzophenone imines together with benzophenone itself (Figure 5.4). These three products were identified with purification and characterization or by reference compounds. To verify that benzophenone is not formed from octocrylene itself when water is present, as it is in the used solvent systems, the corresponding control experiment was performed. Thus, octocrylene was dissolved in the different solvent systems and the reaction mixture was analyzed for the presence of benzophenone without addition of any other reactants. However, no degradation of octocrylene or formation of benzophenone could be seen after 21 h.



Benzophenone-*N*-benzylimine | Benzophenone-N_α-acetyl-L-lysine methyl ester imine | Benzophenone

Figure 5.4. The products detected in the reactivity experiments with octocrylene toward the nucleophiles benzylamine and N_α-acetyl-L-lysine methyl ester. Benzophenone-*N*-benzylimine was formed when benzylamine was used as nucleophile and benzophenone- N_α-acetyl-L-lysine methyl ester imine was formed when N_α-acetyl-L-lysine methyl ester was used as nucleophile. Benzophenone was detected in the experiments with both nucleophiles.

The corresponding imines are probably formed via a retro-aldol condensation reaction (Figure 5.5). Benzophenone is subsequently formed when the imines are hydrolyzed by water, and an equilibrium is established between the imine and benzophenone (Figure 5.5). The reaction is initiated by a Michael attack (Figure 5.5), which should favor the softer sulfur nucleophile (found in cysteine) over the harder nitrogen (found in lysine). However, the next reaction step, which correspond to a retro-aldol reaction, includes a second attack of the

nitrogen on the β-carbon and therefore this reaction is only possible from amines and not thiols (Figure 5.5). Nilsson et al. (*100*) showed that the α,β-unsaturated hapten carvone reacts with benzylamine, but in contrast to what was observed in our experiments, the amine formed an imine via attack on the carbonyl carbon instead of the β-carbon, i.e. a Schiff base formation. Furthermore, these Schiff base formations of α,β-unsaturated carbonyl compounds with amines are usually significantly slower than the Michael addition of thiols (*100, 103, 104*).

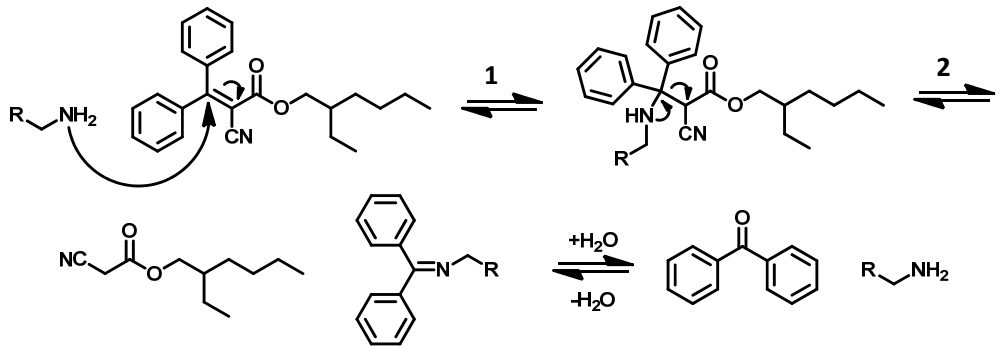


Figure 5.5. Tentative mechanism for the formation of imine adducts and benzophenone when octocrylene reacts with amines. Step 1 is a Michael attack, step 2 is a retro-aldol reaction, and step 1 and 2 together can be seen as a retro-aldol condensation. In presence of water, the formed imines are in equilibrium with benzophenone.

Octocrylene is an α,β-unsaturated carbonyl compound and would therefore be classified as a "Michael-acceptor" hapten by the current structural alert based classification systems used to identify allergens (*103-105*). Although octocrylene behaves as Michael acceptor in the first step of the reaction with nucleophiles, the reaction outcome is not a Michael addition adduct. Octocrylene would, in other words, be assigned to an erroneous mechanistic domain for skin sensitizers by current structural alert based classification systems. Furthermore, octocrylene's reactivity toward amines and thiols highlights the importance of using different kinds of nucleophiles in reactivity experiments for screening of contact allergens. There are examples in the literature were only free thiol is monitored to evaluate the reactivity of α,β-unsaturated carbonyl compound (*101, 102*) and in such an assay octocrylene's reactivity would go undetected.

5.4 Photostability of Octocrylene (Paper III)

There are a number of studies which demonstrate that octocrylene is a very photostable compound (*42-44*). In our photolysis of octocrylene in cyclohexane no degradation could be detected with our HPLC/MS method, even after 6 h of irradiation, and only trace amounts of two products were observed: benzophenone and 3,3-diphenylacrylonitrile (Figure 5.6).

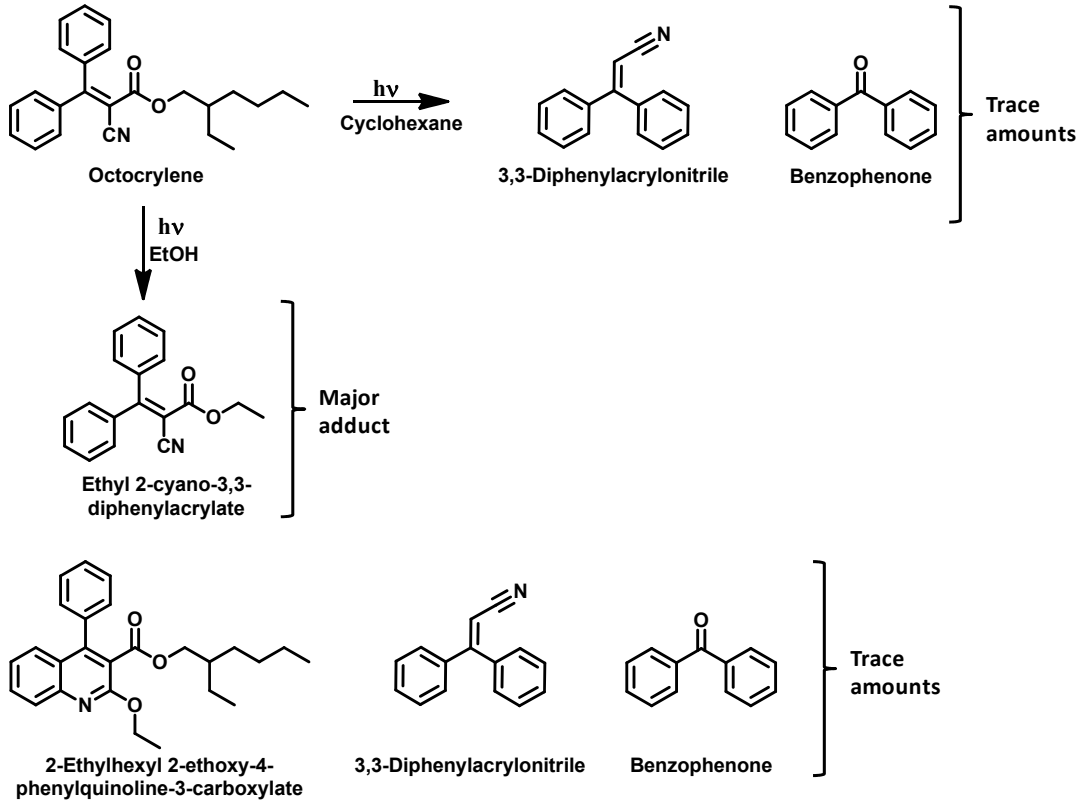


Figure 5.6. Structures of the detected products from the photolysis of octocrylene in cyclohexane and EtOH, respectively.

On the other hand, when EtOH was used as solvent, octocrylene did degrade during the irradiation (Figure 5.7). Also in this photolysis experiment, approximately the same tiny amounts of benzophenone and 3,3-diphenylacrylonitrile was seen. However, the major photoproduct was ethyl 2-cyano-3,3-diphenyl acrylate (Figure 5.6), which is formed via a transesterification of octocrylene with EtOH. A small amount of a fourth product was also detected, and after fractionation of the photolysis mixture this product was characterized as the cyclized product: 2-ethylhexyl 2-ethoxy-4-phenylquinoline-3-carboxylate (Figure 5.6). The formation rate of the major adduct, ethyl 2-cyano-3,3-diphenyl acrylate, was also measured (Figure 5.7).

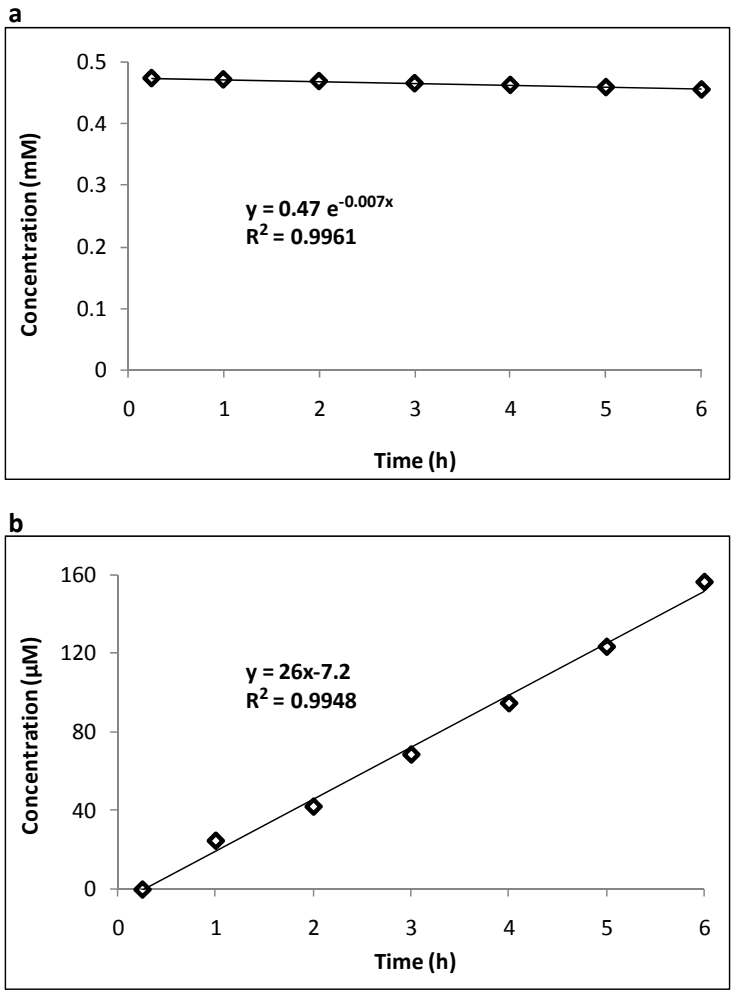


Figure 5.7. (a) Depletion of octocrylene when irradiated in EtOH and (b) formation of the major adduct, ethyl 2-cyano-3,3-diphenyl acrylate, during this experiment.

5.5 Photochemical Reactivity of Octocrylene (Paper III)

To further investigate octocrylene's modified reactivity during irradiation, compared to in absence of UV radiation, additional photochemical reactivity experiments were performed. To simplify the analysis of the photoreaction mixtures, amino acid analogs were considered instead of the amino acids themselves. In total four different amino acid analogs were used, analogs of tyrosine, tryptophan, cysteine and lysine. Analogs of tyrosine and tryptophan were included since these two amino acids are the ones that are most prone to undergo photochemical reactions (*106*). Analogs of cysteine and lysine were included because these are the ones most often considered to be involved in the formation of immunogenic hapten-protein complexes (*27*). Also, octocrylene's ground-state reactivity was studied using these two amino acids (section 4.2.3). As previously mentioned, octocrylene reacted with EtOH during the photolysis, so therefore cyclohexane was chosen as solvent.

As a control experiment the photostability of each individual amino acid analog was investigated before the photochemical reactivity experiments with octocrylene was initiated. The depletion of each analog can be seen in Figure 5.8. Excellent curve fitting was obtained

with a first-order type expression (Figure 5.8), so therefore the half-lives for each amino acid analog were calculated from the exponential factor obtained from the first-order curve fittings (Table 5.3). All amino acid analogs degrade rather fast during the conditions in the photolysis experiments. As expected the tyrosine and tryptophan analogs degraded fastest with half-lives shorter than 15 min, whereas the half-lives for the cysteine and lysine analogs were approximately four times longer (Table 5.3).

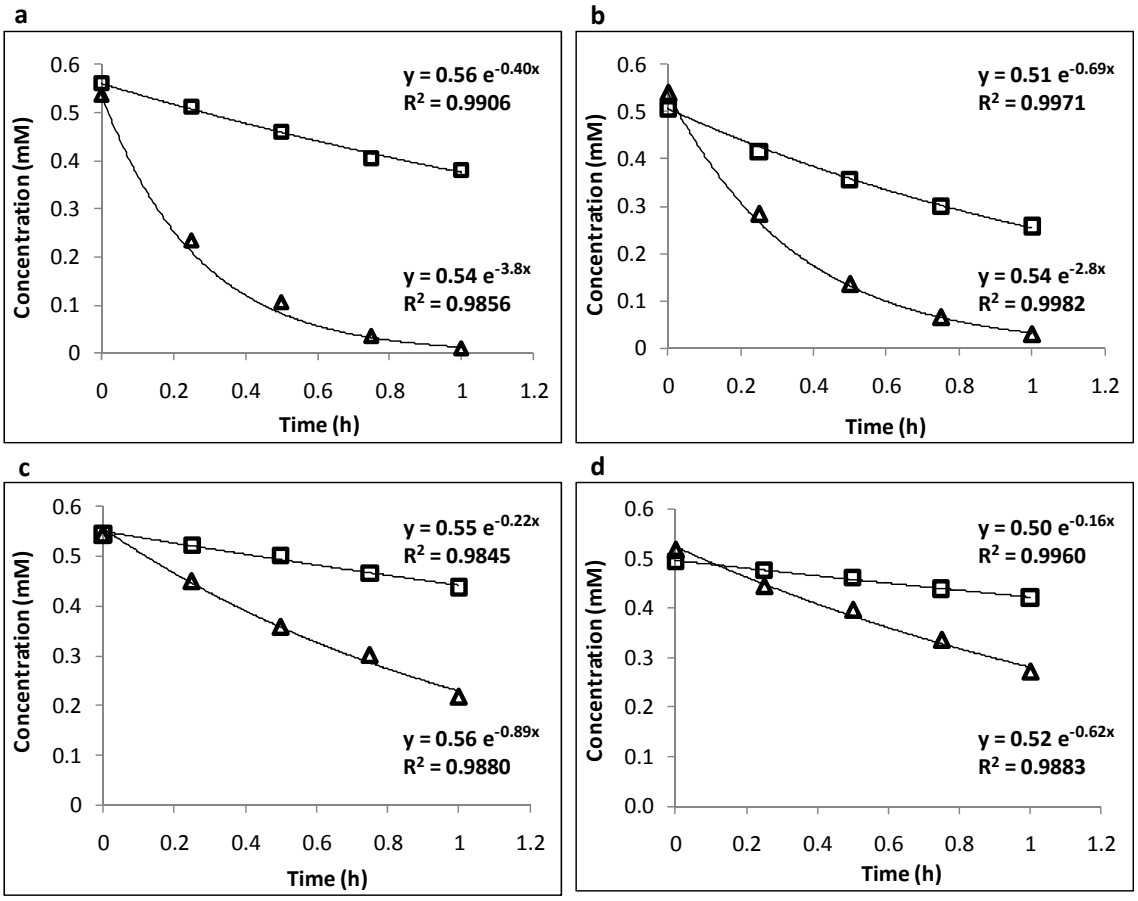


Figure 5.8. Results from photolysis of amino acid analogs with and without octocrylene. Experiments without octocrylene are indicated with the symbol Δ, and experiments with octocrylene in the mixture are indicated with the symbol □. (a) Concentration of the tyrosine analog p-propylphenol with time. (b) Concentration of the tryptophan analog 3-methylindole with time. (c) Concentration of the cysteine analog 1-octanethiol with time. (d) Concentration of the lysine analog benzylamine with time.

The photolysis experiments were then repeated in the same way, but with octocrylene present in the solution. Notably, octocrylene actually seems to do a pretty good job as a UV-filter, since the degradation of all amino acid analogs is significantly slower (Figure 5.8 and Table 5.3). Further, no degradation of octocrylene itself could be observed within the time span of the experiment.

Table 5.3. Half-lives[a] for the amino acid analogs in the presence and absence of octocrylene

compound	octocrylene	$t_{1/2}$ (h)
Tyr-analog[b]	no	0.21 ± 0.02
Tyr-analog[b]	yes	1.7 ± 0.1
Trp-analog[b]	no	0.24 ± 0.01
Trp-analog[b]	yes	0.85 ± 0.1
Cys-analog[c]	no	0.78
Cys-analog[c]	yes	3.2
Lys-analog[c]	no	1.1
Lys-analog[c]	yes	4.3

[a] $t_{1/2} = \frac{ln2}{k}$, for firs-order reactions where $[A]=[A]_0\, e^{-kt}$
[b] $t_{1/2}$ is based on two experiments.
[c] $t_{1/2}$ is based on one experiment.

Surprisingly, when octocrylene is in the solution, the half-life of the tyrosine analog is eight times longer, whereas the half-lives for the other three analogs are approximately four times longer. In order to understand why this is, it is necessary to understand how octocrylene can protect the amino acid analog from degradation. The following equations can be used to describe the degradation of amino acid analogs, with and without octocrylene:

$$A + h\nu \rightarrow A^* \quad (5.1)$$

$$A^* \rightarrow I \rightarrow DP \quad (5.2)$$

$$OCT + h\nu \rightarrow OCT^* \quad (5.3)$$

$$A^* + OCT \rightarrow A + OCT^* \quad (5.4)$$

Irradiation of the amino acid analog, in its ground state (A), promotes it to its excited state (A*) (Eq. 5.1). The excited amino acid analog (A*) can then either return to its ground state, via emission and internal conversion, or form degradation products (DP) via one or several reactive intermediates (I) (Eq. 5.2). Octocrylene's (OCT) protective ability is due to two things: firstly, it can absorb some of the UV radiation (Eq. 5.3) and thereby can shield the amino acid analog from the harmful light and secondly, octocrylene can quench the excited state of the amino acid analog decreasing the available time for it to react (Eq. 5.4) (*44, 107*). In other words, there are two reasons for the observed decrease in degradation rate of the amino acid analogs: less amino acid analog is being excited and the quenching process competes with the photochemical degradation. Our first hypothesis to why the tyrosine analog is protected more from photodegradation by octocrylene than the other amino acid analogs was that the tyrosine analog has a larger spectral overlap with octocrylene. However, comparison between the UV absorption spectrum of each amino acid analog and that of octocrylene, in the same concentrations in cyclohexane, did not show and significant difference in spectral overlap between the absorption spectra of octocrylene and the different analogs.

No reaction products were detected in the photolysis experiments with octocrylene and the amino acid analogs for tyrosine, tryptophan and cysteine. However, in the photochemical experiment with benzylamine (the lysine analog) the formation of a reaction product was

observed, and fascinatingly, this adduct was not the benzophenone-*N*-benzylimine that was formed in the corresponding chemical experiments (see section 4.2.3 and Figure 5.9). Instead, after fractionation of the mixture and characterization, the reaction product was shown to be the amide *N*-benzyl-2-cyano-3,3-diphenylacrylamide, formed from a reaction of benzylamine with the carbonyl carbon of octocrylene (Figure 5.9).

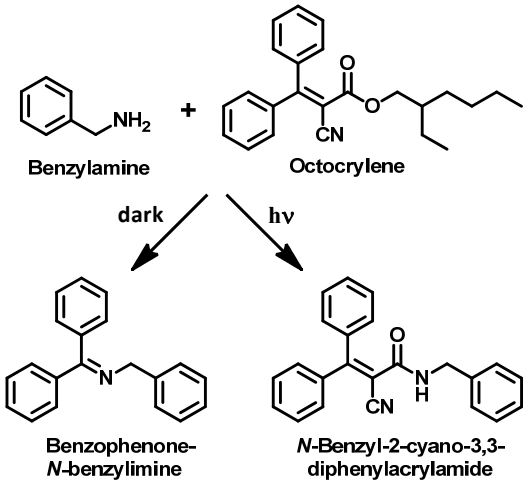


Figure 5.9. Structures of the different products formed between benzylamine and octocrylene with and without UV-light.

To further study this photochemical reaction between octocrylene and benzylamine, a series of photolysis experiments with a fixed octocrylene concentration (10 mM) and varying benzylamine concentrations (0.25 to 4 equivalents) was performed. Interestingly, there was no difference in the formation rate of the *N*-benzyl-2-cyano-3,3-diphenylacrylamide (Figure 5.10). This amidation reaction is equivalent to the transesterification observed in the photostability experiment of octocrylene in EtOH (Figure 5.6). Furthermore, the formation rate of the two products (*N*-benzyl-2-cyano-3,3-diphenylacrylamide and ethyl 2-cyano-3,3-diphenyl acrylate) is in the same range (Figure 5.7), which further supports a monomolecular rate limiting step in the reaction sequence to the amide.

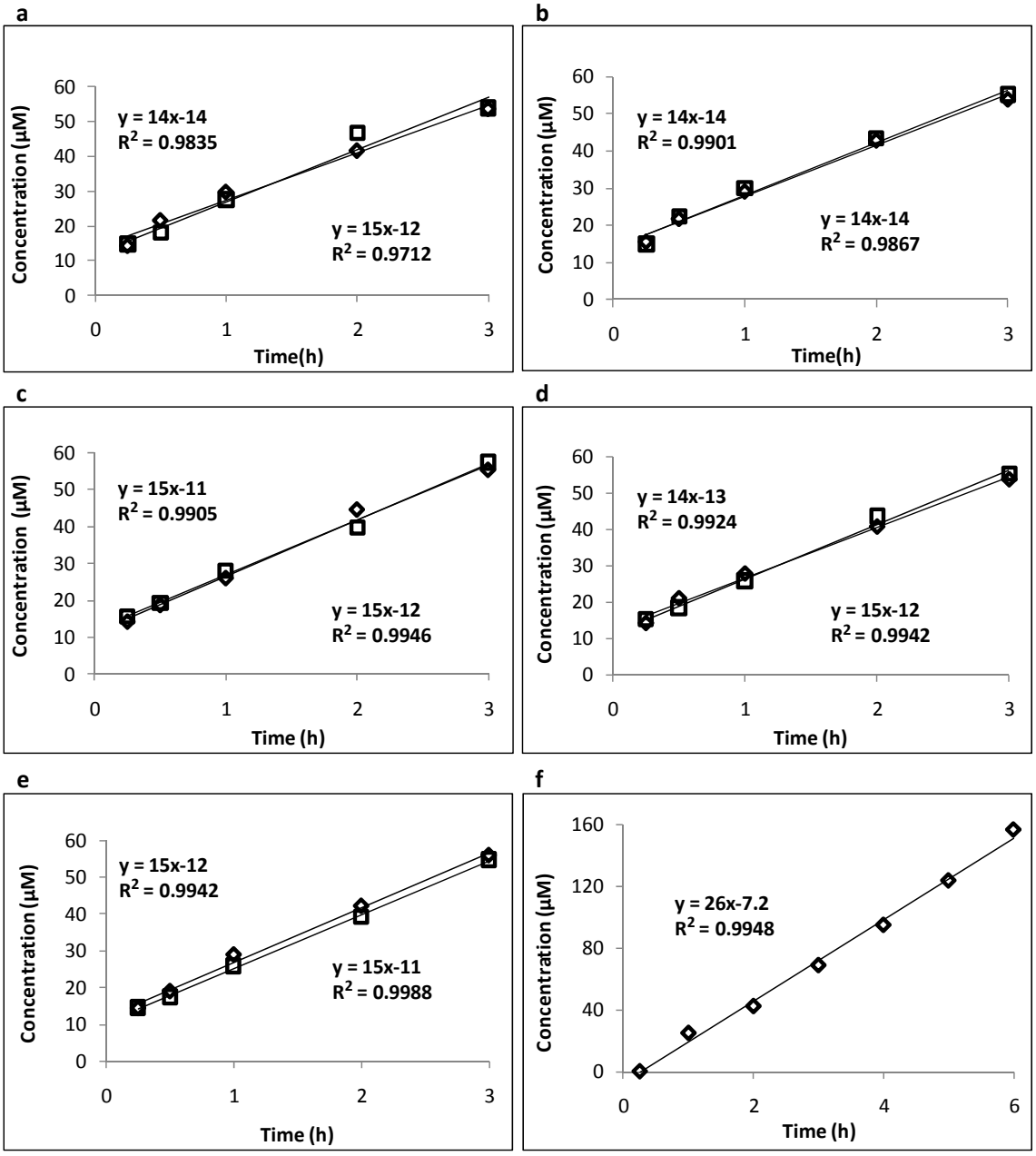


Figure 5.10. Formation of the UV-induced reaction product *N*-benzyl-2-cyano-3,3-diphenylacrylamide, in concentration with time, when the concentration of octocrylene is 10 mM and the concentration of benzylamine is varied: (a) 2.5 mM, (b) 5 mM, (c) 10 mM, (d) 20 mM, and (e) 40 mM. Two experiments were performed for each benzylamine concentration. For comparison, the formation of the major adduct from the photolysis of octocrylene in EtOH, ethyl 2-cyano-3,3-diphenyl acrylate, is shown in the last picture (f).

5.6 Concluding Discussion

Octocrylene has in the last years become one of the most frequently observed photocontact allergens in the clinics. However, both our clinical study as well as other studies have shown that octocrylene causes both photocontact allergy (60-80%) and contact allergy (20-40%). Until now, no studies have been published that investigates the chemical properties of octocrylene, thus explaining both of these adverse effects. In general, the mechanisms behind photocontact allergy are much less explored than those involved in contact allergy. Therefore, we first aimed at understanding which structural features of octcorylene make it into a contact allergen. The assessment of octocrylene in the LLNA revealed that it is a moderate sensitizer in the murine model and our chemical reactivity experiments showed that octocrylene forms stable adducts with benzylamine and lysine, but not with cysteine. Octocrylene reacts with these amines via a retro-aldol condensation, which results in the formation of the corresponding benzophenone imines. There are a few examples in the literature where similar reactions of α,β-unsaturated carbonyls with amines have been reported (*108, 109*). However, a retro-aldol condensation has, to the best of our knowledge, never before been suggested as a mechanistic pathway to the formation of immunogenic hapten-protein complexes. Octocrylene's reactivity toward amines, which in the body is mainly found in the form of lysine residues, is the probable explanation for its ability to induce contact allergy. Moreover, the fact that mostly children display contact allergy to octocrylene could be because they generally are subjected to larger amounts of octocrylene, due to more frequent application of sunscreen. Also, many of the sunscreens on the market that are intended for children contain high concentrations of octocrylene in order to obtain a high enough sun protection factor (SPF). Another important factor is that children have a larger body surface area to body weight than adults.

After having established the probable reason for octocrylene's contact allergenic properties, we moved on to study its photostability and reactivity in presence of UV radiation. The objective was to provide more knowledge concerning the mechanism behind its photocontact allergenic properties. In agreement with other studies we found that octocrylene is highly photostable. However, when EtOH was used as solvent in the photolysis experiment, octocrylene did react with the solvent in a transesterification reaction, which gave ethyl 2-cyano-3,3-diphenyl acrylate as a product. EtOH can be seen as a model for primary alcohols, which can be found in skin proteins in the form of serine residues. When the photochemical reactivity of octocrylene was studied, we chose analogs for the amino acids tyrosine, tryptophan, cysteine and lysine. The presence of octocrylene was shown to slow down the photodegradation of all four amino acid analogs. However, more interestingly, octocrylene did react with the lysine analog, benzylamine, but via a different mechanism than in the absence of UV radiation. The formed product was *N*-benzyl-2-cyano-3,3-diphenylacrylamide, i.e. an amide formation takes place. In other words, this is the corresponding reaction to the transesterification that took place when octocrylene was irradiated in EtOH. In addition, our results indicate that this acylation reaction is independent of the concentration of the amine or the alcohol. The fact that octocrylene in the presence of light reacts via a different reaction pathway that outcompetes the dark reaction may explain the higher incidence of photocontact allergy, compared to contact allergy. Further, the difference in the hapten-protein complexes that octocrylene may form in its excited state compared to in its ground state could explain why photocontact allergic patients usually do not present a positive reaction in the absence of UV-light.

In our clinical study, as well as in other studies, it is notable that many of the patients that suffer from photocontact allergy to octocrylene have a history of topical ketoprofen use. It therefore seems as if photocontact allergy to ketoprofen also leads to photosensitization to octocrylene. Unfortunately, patients that have experienced skin reactions to sunscreens, but who have never used topical ketoprofen are generally not tested with ketoprofen. Therefore, it is not known whether octocrylene can induce photocontact allergy to ketoprofen, or if it only is the other way around. However, none of the photoproducts that octocrylene formed with EtOH and benzylamine could be formed from ketoprofen. The major photodegradation product formed from ketoprofen, in neutral aqueous solutions, is 3-ethyl benzophenone (*110, 111*), the reaction product formed between amines and ground state octocrylene corresponds to a Schiff base formation of benzophenone and the amine, and benzophenone-3 is a benzophenone derivative (Figure 5.11).

Figure 5.11. Structures of three different benzophenone derivatives.

At first it may therefore seem as if benzophenone could be a common denominator in these apparent photocross reactions to ketoprofen and octocrylene. However, if this should have been the case, the ketoprofen photoallergic patients should react to octocrylene without UV radiation, which they seldom do. Therefore, our hypothesis is that octocrylene can cause allergic reactions via three different pathways: the first route is via octocrylene's ground state reactivity toward amines, the second possibility is through octocrylene's excited state reactivity toward amines and alcohols, and finally a third pathway that generates an immunogenic complex that can also be formed in the presence of ketoprofen and UV radiation. This theory results in three different groups of octocrylene allergic patients: the first group contains contact allergic patients, the second group consists of patients with photocontact allergy to octocrylene but with no allergy to ketoprofen, and the last group includes patients with photocontact allergy to both octocrylene and ketoprofen. The reports, so far, from the clinics seem to support the existence of the first and the last group of octocrylene allergic patients. In order to find out if there are also patients that display photocontact allergy to octocrylene but not to ketoprofen, more clinical studies are needed where patients are tested simultaneously for photocontact allergy to both compounds, regardless of their anamnesis.

6 STUDIES OF KETOPROFEN (PAPER IV)

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6.1 Photostability of Ketoprofen

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6.2 Photolysis of Amino Acid Analogs in Presence of Ketoprofen

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6.3 Concluding Discussion

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7 SYNTHESIS OF SCYTONEMIN (PAPER V)

In the search for new pharmacophores, natural products are of considerable value. We therefore searched the literature for natural products that may serve as templates in the development of new chromophores that could be used as more efficient chemical UV-filters than the ones that are on the market today. Cyanobacteria have the ability to survive in places such as desert soils, rocks, and shallow marine intertidal areas were the solar UV radiation is extremely intense (*14, 119*). Their ability to survive has been assigned to the photostable pigment scytonemin (Figure 7.1), which absorbs the UV radiation (*14, 16*). Scytonemin's highly conjugated, homodimeric skeleton is unique among natural products and its heterocyclic ring system has been given the trivial name scytoneman (Figure 7.1) (*15*). The reduced form of scytonemin (Figure 7.1) has also been found in cyanobacteria that have become buried in sediments and in cells that are no longer viable (*14*).

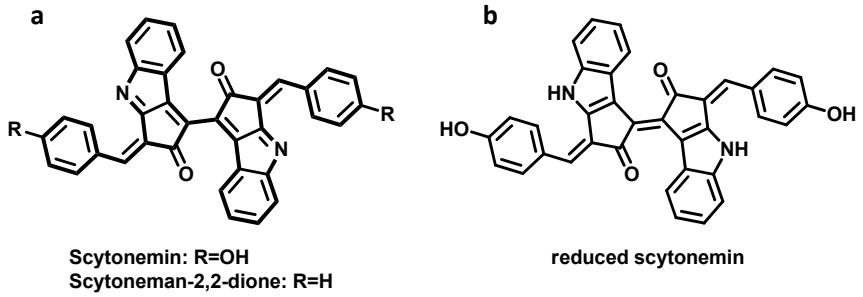
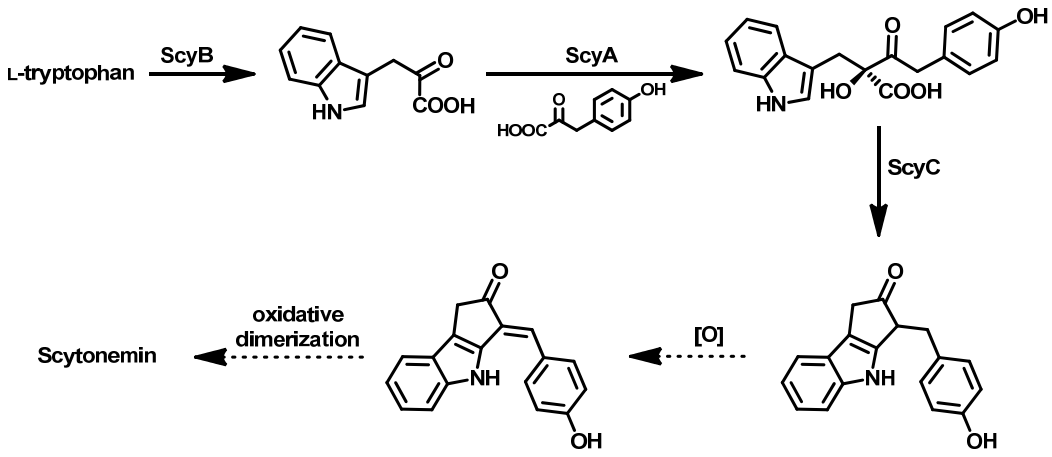


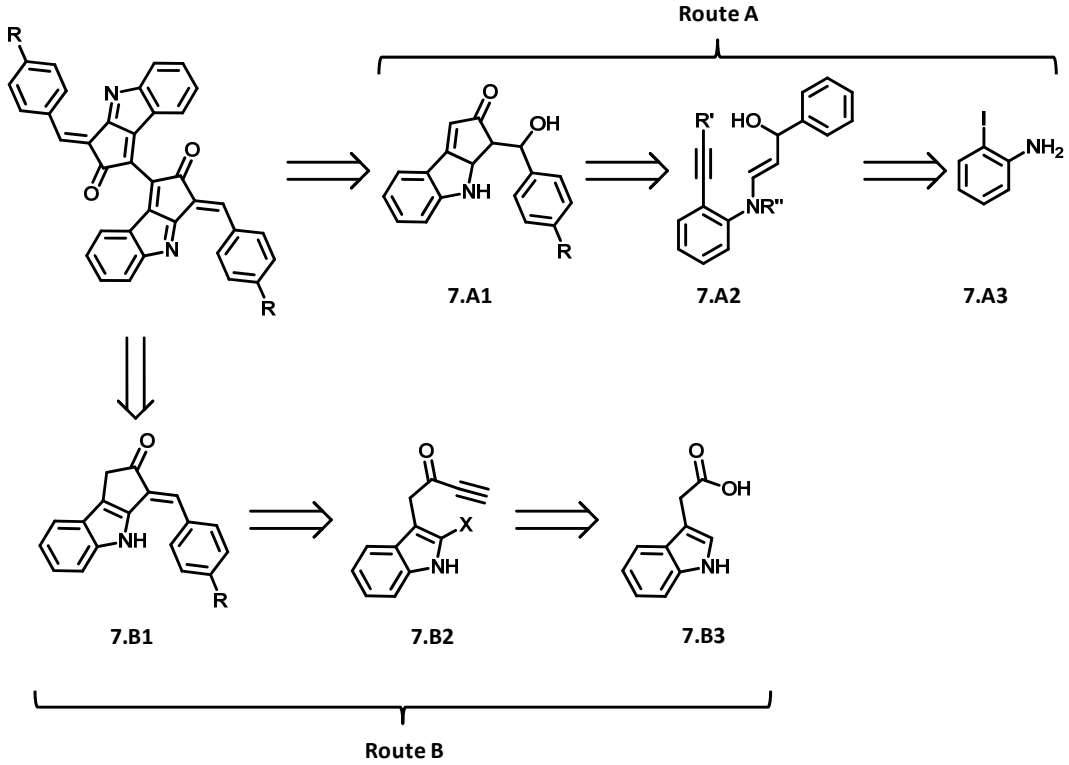
Figure 7.1. (a) Structures of scytonemin and its derivative scytoneman-2,2-dione. The scytoneman skeleton is highlighted by bold lines. (b) Structure of the reduced form of scytonemin.

The biosynthesis of scytonemin has been proposed to start from L-tryptophan. In the initial step of this biosynthetic pathway L-tryptophan is converted to 3-indole pyruvic acid by the enzyme ScyB. In the following step a β-ketoacid is formed by coupling of the 3-indole pyruvic acid with *para*-hydroxyphenylpyruic acid, promoted by the enzyme ScyA. The β-ketoacid is thereafter cyclized by the enzyme ScyC, which produces the reduced form of the scytonemin monomer. Finally, Scytonemin is thought to be formed via oxidative dimerization of this reduced scytonemin monomer (Scheme 7.1) (*120, 121*).



Scheme 7.1. Proposed biosynthetic pathway for scytonemin. The dashed arrows indicate unexplored reactions.

We wanted to study the photochemical and photophysical properties of scytonemin to understand which structural elements are responsible for its photostability. However, to do this, we first had to develop a synthetic route to this natural product. After retrosynthetic analysis, two different routes (A and B) to access scytonemin was proposed, see Scheme 7.2. The key step in each route is a cyclization reaction. In route A the plan was to obtain the key intermediate **7.A1** from **7.A2** via a Pauson-Khand reaction, and in route B the aim was to assemble the key intermediate **7.B1** from **7.B2** via a tandem Heck-Suzuki-Miyaura reaction. Because the phenolic group was predicted to be problematic, to explore the viability of the planned routes it was decided that the first synthetic target should be scytonemin derivative with a phenyl group instead of a 4-hydroxyphenyl group (scytoneman-2,2'-dione) (Figure 7.1).

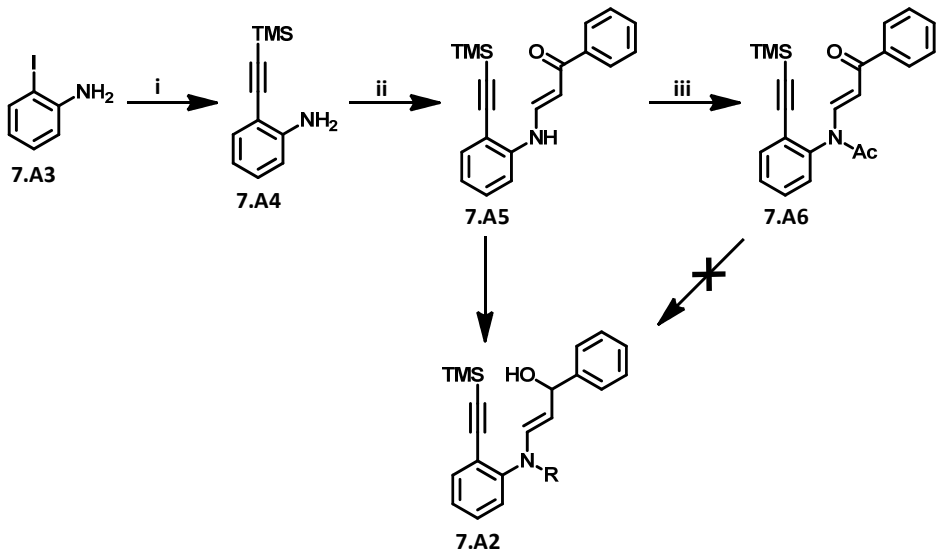


Scheme 7.2. The two proposed routes to scytonemin after retrosynthetic analysis. Route A starts from 2-iodoaniline (**7.A3**), whereas route B starts from 3-indole acetic acid (**7.B3**).

7.1 Synthetic Route A to Scytoneman-2,2-dione

The key step in the first proposed synthetic route to the scytonemin derivative scytoneman-2,2-dione is the Pauson-Khand cyclization. In general, the Pauson-Khand reaction works with a wide-variety of substituents on the alkyne. However, electron-deficient alkenes are known to be poor substrates, whereas Pauson-Khand reactions with electron-donating substituents on the alkene generally afford the desired products (*122*). The enyne **7.A2** was therefore considered to be a suitable substrate for this cyclization reaction and thereby became the first synthetic target. The explored route to synthesize the enyne **7.A2** from 2-iodoanline (**7.A3**) is shown in Scheme 7.3; detailed experimental procedures can be found in Appendix II. The α,β-unsaturated ketone **7.A5** was obtained via a Sonogashira coupling between **7.A3**

and TMS-acetylene (*123*) followed by a Michael addition of aniline **7.A4** to 1-phenyl-2-propyn-1-one (*124*). In the following step it was desirable to reduce the carbonyl functionality before the subsequent ring-closing Pauson-Khand reaction to obtain an electron-rich alkene. In other words, the last step before the Pauson-Khand substrate **7.A2** was obtained was a reduction of the ketone in compound **7.A5**. However, this step turned out to be much harder than first anticipated.



7.A3 7.A4 7.A5 7.A6 7.A2

Scheme 7.3. Explored procedure to obtain compound **7.A2**. Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$ (0.05 equiv), CuI (0.05 equiv), TMS-acetylene (1.1 equiv), Et_3N (3 equiv), RT, 18h, 97%. (ii) 1-Phenyl-2-propyn-1-one (1 equiv), toluene, 90 °C, 64%. (iii) AcCl (1.25 equiv), DMAP (cat amount), Et_3N (1.25 equiv), DCM, reflux, 5h, 90%.

Reductions of carbonyl compounds are most often done with reagents that can transfer a hydride ion to the carbonyl carbon. The two most commonly employed reducing agents are $NaBH_4$ and $LiAlH_4$. $NaBH_4$, which is the weaker reducing agent of the two, can generally be used to reduce aldehydes and ketones, but not esters or amides. $LiAlH_4$, on the other hand, is known to reduce all of these carbonyl groups. However, $LiAlH_4$ is not as easy to work with as $NaBH_4$, so therefore $NaBH_4$ is often employed when possible. The substrate that we wanted to reduce is an α,β-unsaturated ketone and reduction of such compounds with $NaBH_4$ or $LiAlH_4$ can result in both 1,2- or 1,4-reductions. Furthermore, in a substrate where 1,4-reduction is favored over 1,2-reduction it is also common to observe over reduction, i.e. the alkene is reduced first in a 1,4-reductions and then the ketone a 1,2-reduction, resulting in a saturated alcohol. In the 1970s Luche developed a procedure for such substrates where 1,2-reduction is favored over 1,4-reduction (*125*). This procedure uses $NaBH_4$ or $LiAlH_4$ together with the hard Lewis acidic metal cerium, which coordinates to the carbonyl oxygen and thereby enhances the electrophilicty of the carbonyl carbon.

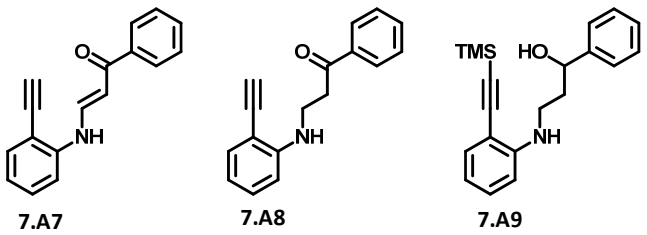
No literature procedure could be found where the carbonyl of an *N*-aryl-enaminone had been reduced. However, a few articles were found in which successful 1,2-reductions had been made of other enaminones (*126-130*). All of these procedures used $NaBH_4$ as reducing agent and most of them used it in combination with $CeCl_3$, i.e. the so called Luche conditions. The different procedures that were explored in order to reduce the ketones **7.A5** and **7.A6** are summarized in Table 7.1. In the first experiment $NaBH_4$ was used as reducing agent and MeOH was used as solvent (Table 7.1, entry 1), but only starting material was

recovered. The substrate did not dissolve properly in this first attempt, therefore the solvent was changed to MeOH/toluene (1:1) in the following experiment (Table 7.1, entry 2). However, this reaction only resulted in TMS deprotected starting material (**7.A7**), see Figure 7.2. In the following attempt the Luche conditions were tested. Unfortunately, these conditions did not work either and the starting material was recovered (Table 7.1, entry 3).

Table 7.1. Result from the different reduction procedures that were tested.

entry	substr	red agent	Lewis acid	solvent	result
1	**7.A5**	$NaBH_4$	-	MeOH	no reaction
2	**7.A5**	$NaBH_4$	-	MeOH/Toluene (1:1)	TMS depr (compd **7.A7**)
3	**7.A5**	$NaBH_4$	$CeCl_3 \cdot 7H_2O$	THF	no reaction
4	**7.A7**	$LiAlH_4$	-	THF	alkene red (compd **7.A8**)
5	**7.A5**	$LiAlH_4$	$CeCl_3 \cdot 7H_2O$	THF	no reaction
6	**7.A5**	$LiAlH_4$	$CeCl_3$ anhyd	THF	over red (compd **7.A9**)
7	**7.A6**	$LiAlH_4$	$CeCl_3$ anhyd	THF	Ac depr (compd **7.A5**)

We next tried the much stronger reducing agent $LiAlH_4$. The first attempt was with the *N*-aryl enaminone **7.A7** without the TMS group as starting material instead of the TMS protected *N*-aryl enaminone **7.A5**. However, the obtained product was the saturated ketone **7.A8** in which a 1,4-reduction had taken place instead of a 1,2-reduction, see Figure 7.2 and Table 7.1, entry 4. Even if it was not the desired bond that was reduced, the fact that a reduction had occurred was a good sign. The next attempt to reduce the ketone was therefore with $LiAlH_4$ together with $CeCl_3$, which should favor the 1,2-reduction over the 1,4-reduction. Surprisingly, in principle only starting material was recovered from this reaction (Table 7.1, entry 5). It was suspected that the $LiAlH_4$ might have degraded. Therefore, the reaction was repeated with fresh $LiAlH_4$ and anhydrous $CeCl_3$ instead of the hepta hydrate (Table 7.1, entry 6), but these conditions only gave the over reduced compound **7.A9** (Figure 7.2).



7.A7 **7.A8** **7.A9**

Figure 7.2. Structures of compounds A7-A9.

A possible explanation to why neither of these reductions worked is that the equilibrium between the α,β-unsaturated ketone **7.A5** and the α,β-unsaturated imine **7.A5'** is largely shifted to the imine, which would give a more conjugated system (Figure 7.3). However, both the ^{1}H-NMR and ^{13}C-NMR shifts seem to agree better with the ketone form than the imine form (Appendix II).

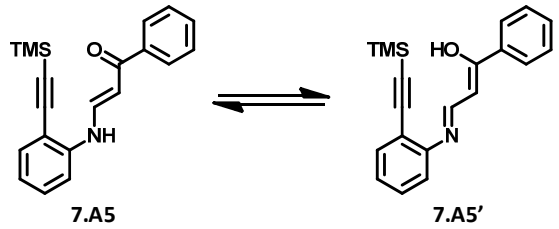
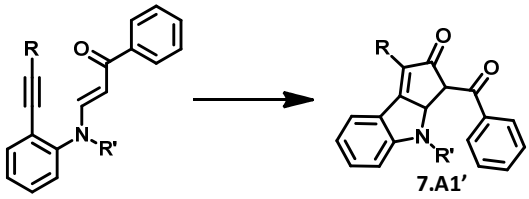


Figure 7.3. Keto-enol tautomerism between the ketone 7.A5 and the imine 7.A5'.

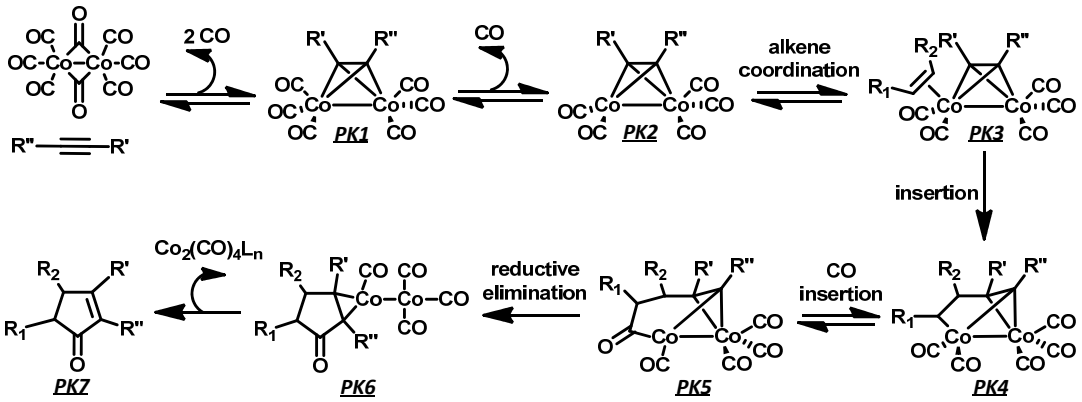
In an attempt to circumvent this problem, the amine in α,β-unsaturated ketone **7.A5** was acetyl protected (Scheme 7.3) and a new experiment to reduce the ketone, with $NaBH_4$ and anhydrous $CeCl_3$, was performed (Table 7.1, entry 7). However, this reaction only gave the starting material (**7.A6**) together with the acetyl deprotected compound **7.A5**.

After these seven unsuccessful attempts, it was decided that it was not possible to spend more time on trying to obtain compound **7.A2** via this route. Although, electron-deficient alkenes are considered to be unsuitable substrates for the Pauson-Khand reaction, there are a few examples of successful Pauson-Khand reactions with α,β-unsaturated carbonyl compounds as substrates (*131, 132*). We therefore decided to try the intramolecular Pauson-Khand reaction with the unreduced α,β-unsaturated ketones **7.A5**-**7.A7**, which if succeeded would result in the diketone **7.A1'** instead of **7.A1** (Scheme 7.4).



Scheme 7.4. Planed intramolecular Pauson-Khand reaction to obtain the cyclized compound **7.A1'**.

The cobalt-mediated Pauson-Khand reaction was discovered in the 1970s (*133, 134*). In this reaction a α,β-cyclopentenone is formed via a [2+2+1] cycloaddition of an alkyne, an alkene and carbon monoxide (CO). The cobalt complex used is a dicobalt octacarbonyl complex (Scheme 7.5). The widely accepted Pauson-Khand mechanism is described in Scheme 7.5 (*135-138*). It starts with the replacement of two CO molecules by the alkyne, which generates the cobalt-alkyne complex PK1. The following ligand dissociation step, generation of PK2, is a decarbonylation and this can be achieved either thermally or oxidatively. Thereafter comes the coordination of the alkene (PK3) followed by the irreversible insertion of the alkene between the cobalt and one of the alkyne carbons, which gives complex PK4. In the next step, a carbonyl is inserted in the newly formed bond between one of the alkene carbons and cobalt (PK5). Finally a reductive elimination gives the product, first as a cobalt complex (PK6) and eventually as the α,β-cyclopentenone (PK7).



Scheme 7.5. Proposed mechanistic pathway for the Pauson-Khand reaction.

In the first two attempts, so called mild Pauson-Khand conditions, i.e. room temperature and addition of trimethylamine *N*-oxide (TMANO) as a promoter for the decarbonylation step (*139*), were tested on both the unprotected and TMS protected α,β-unsaturated ketone **7.A5** and **7.A7**. However, only starting material was recovered from both reactions (entry 1-2, Table 7.2). In the next attempt (entry 3, Table 7.2), the *N*-acetyl protected **7.A6** was used as starting material and slightly more harsh thermal conditions were used (70 °C and no addition of TMANO), but the only recovered compound was the *N*-aryl enaminone **7.A5**, i.e. the acetyl group had been cleaved off.

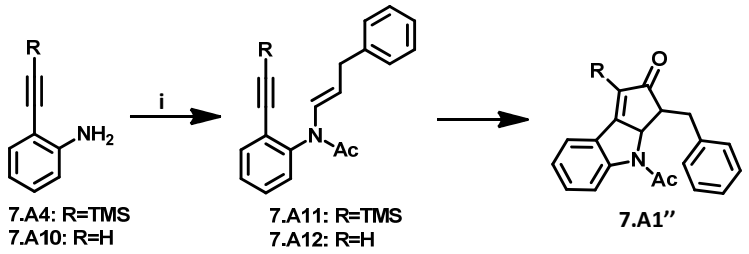
Table 7.2. Result from the different Pauson-Khand conditions that were tested.

entry	substr	temp	additive	solvent	Result
1	**7.A5**	rt	TMANO	Toluene	no reaction[a]
2	**7.A7**	rt	TMANO	Toluene	no reaction
3	**7.A6**	70 °C	-	Toluene	Ac depr
4	**7.A11**	reflux	DodMeS	1,2-DCE	no reaction
5	**7.A12**	rt	TMANO	Toluene	no reaction
6	**7.A12**	reflux	-	Toluene	Trace amounts[b]
7	**7.A12**	60 °C	-	Toluene	Complex mix[c]

[a]Starting material and TMS deprotected starting material was recovered.
[b]A complex mixture was obtained and one compound had the right mass according to GC/MS. The major products are believed to be **7.A13** and **7.A14**.
[c]A mixture of several compounds was obtained. Compounds **7.A13** and **7.A14** were identified but no compound with the desired mass could be detected with the GC/MS.

As already mentioned, electron-deficient alkenes, such as α,β-unsaturated carbonyls, are not suitable substrates for Pauson-Khand reactions (*122*). However, the vinylic nitrogen on the α,β-unsaturated system should to some degree compensate for the electron withdrawing effect of the carbonyl group. Acetylation of the vinylic nitrogen on the other hand should decrease this mesomeric effect and result in a less active substrate. To get a more active substrate for the Pauson-Khand reaction, a compound with a CH_2-group instead of the ketone, i.e. the corresponding enamide, was prepared. A literature procedure was used to prepare the enamides **7.A11** and **7.A12** in a one-pot reaction (*140*) starting with addition of hydrocinnamaldehyde to aniline **7.A4** or **7.A10** followed by addition of acetyl chloride to trap

the desired enamide **7.A11** or **7.A12**, respectively (Scheme 7.6); a detailed experimental procedure can be found in Appendix II. These two compounds (**7.A11** and **7.A12**) were then evaluated in the Pauson-Khand reaction, which would give compound **7.A1''** if the reaction succeeded (Scheme 7.6).



Scheme 7.6. Synthesis of compounds **7.A11** and **7.A12** and the structure of the compound **7.A1''** that would be obtained if the subsequent Pauson-Khand reaction would succeed. Reagents and conditions: (i) 1) hydrocinnamaldehyde (1.2 equiv), THF, -10 °C to RT, 4.5 h; 2) AcCl (1.05 equiv), Et_3N (1.2 equiv), RT, 1h.

The next Pauson-Khand attempt (entry 4, Table 7.2) was with the TMS protected *N*-acetyl enamine **7.A11** as starting material and a slightly different experimental procedure. There are examples in the literature were Pauson-Khand reactions with sulfide additives turned out well when other conditions either failed or gave low yields (*141*). Therefore, a procedure was tested where *n*-dodecyl methyl sulfide (DodSMe) is used as promoter and 1,2-dichloroethane is used as solvent (*142*). Unfortunately, no reaction was observed when these conditions were used. We then went back to the thermally mild conditions, were TMANO is used as additive, on the *N*-acetyl enamine **7.A12**, but no reaction was observed here either (entry 5, Table 7.2). In the next attempt, also with **7.A12** as starting material, reflux instead of adding TMANO was tested and for the first time cyclization of the substrate seemed to take place. No starting material was recovered from this reaction; instead a complex mixture of several compounds was obtained and one compound had the right mass (according to GC/MS). Unfortunately this compound was formed in such a small amount that it was not possible to isolate enough material to characterize it. The two major reaction products, **7.A13** and **7.A14**, were isolated and based on analysis of the NMR spectra they appear to be monocyclized products without insertion of carbon monoxide (Figure 7.5).

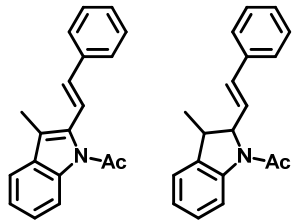
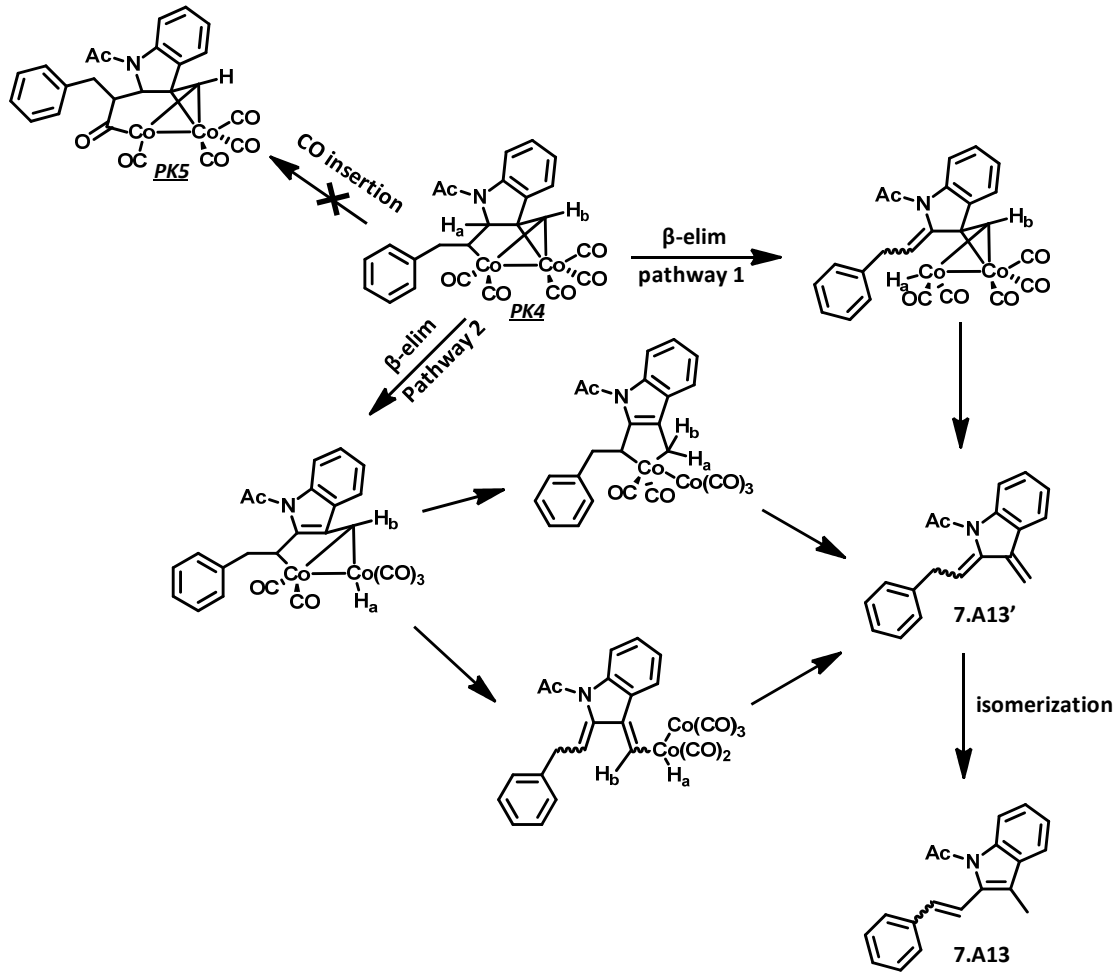


Figure 7.5. Structures of compounds **7.A13** and **7.A14**, which are believed to be formed in the Pauson-Khand reaction of compound **7.A12**.

The diene **7.A13** is probably formed because β-elimination takes place instead of carbon monoxide insertion (Scheme 7.7) (*143*). This reaction pathway will give the diene **7.A13'** that isomerizes into the more stable **7.A13** (Scheme 7.7). The other major reaction product, **7.A14**, is probably formed via some kind of reductive cyclization pathway. However, the mechanism for this reaction is not known. In the literature there are examples where

formation of dienes like **7.A13** have been suppressed in favor of the desired carbonyl compound by using milder thermal conditions (*144*). In a final attempt, the temperature was therefore reduced from reflux to 60 °C. However, also in this case a complex mixture containing both compound **7.A13** and **7.A14** was obtained, but no peak with the correct mass could be detected when the mixture was analyzed with GC/MS.

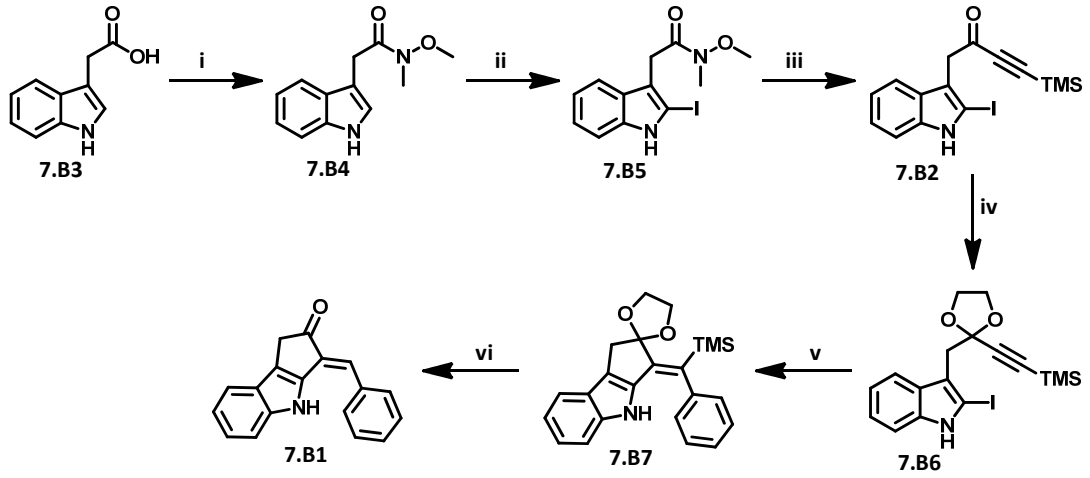
After these seven attempts to promote the Pauson-Khand reaction it was decided that the chances of obtaining compound **7.A1**, or any of its derivatives (**7.A1'** and **7.A1''**), in reasonable yield via route A was low. Therefore, this route was abandoned and route B was explored instead.



Scheme 7.7. The proposed mechanism for the formation of diene **7.A13**. Instead of carbon monoxide insertion (PK4→PK5), a β-elimination takes place, which results in diene **7.A13'** that subsequently isomerizes into **7.A13**.

7.2 Synthetic Route B to scytoneman-2,2-dione

The second synthetic route to scytonemin and its derivative scytoneman-2,2-dione was influenced by scytonemin's proposed biosynthesis (*120, 121*). The key intermediate **7.B1** in this route is the reduced scytonemin (or scytoneman-2,2-dione) monomer that is suggested to be dimerized in the final step of the biosynthesis of scytonemin. The proposed synthetic procedure to obtain compound **7.B1** is shown in Scheme 7.8.



Scheme 7.8. Overview of the explored synthetic route to compound **7.B1**. Reagents and conditions: (i) NMM (1.02 equiv), *i*pr-chloroformate (1.07 equiv), THF, -20 °C, 30 min; 2) Et_3N (1.14 equiv), NHMe(OMe)·HCl (1.23 equiv), DMF, 0 °C, 1h, 81%. (ii) I_2 (0.97 equiv), AgOTf (1.1 equiv), THF, RT, 5 min, 78%. (iii) 1) *n*BuLi (4.2 equiv), TMS-acetylene (4.8 equiv), THF, -78 °C to 10 °C, 2h, 98%. (iv) See Table 7.3. (v) See Table 7.4. (vi) 1) HCl (5 M, aq), acetone, RT, 30 min, 95%; 2) TBAF (2 equiv), THF, 0 °C, 30 min, 99%.

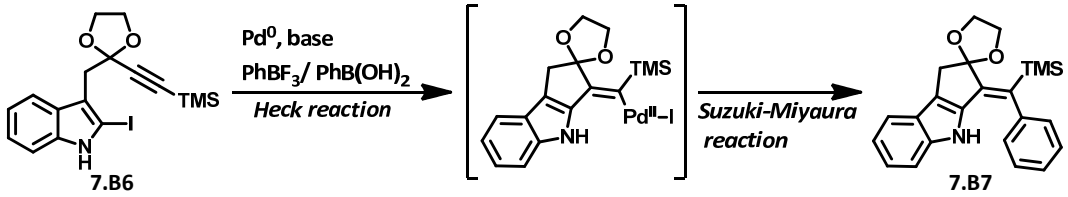
In the first step the commercially available 3-indole acetic acid (**7.B3**) was transformed into the corresponding Weinreb amide **7.B4** by using standard conditions (*145*). In the next step compound **7.B4** was iodinated by using molecular iodine and silver triflate (*146*). The first attempt of this reaction only gave a 50% yield. It was realized that addition of silver triflate to a solution of substrate **7.B4** and iodine substantially increased the yield compared to when iodine was added to a solution of substrate **7.B4** and silver triflate. Another reason to the moderate yield was purification problems, since a diiodinated byproduct was also formed. Careful addition of the silver triflate together with a decrease in the iodine amount to slightly less than one equivalent prevented the formation of the diiiodinated byproduct. Together these modifications increased the yield to approximately 80%. The alkynyl ketone **7.B2** was formed by treating the iodinated Weinreb amide **7.B5** with lithiated TMS-acetylene. It was found in the literature that alkynes with electron-withdrawing groups directly on the triple bond could give poor results in palladium catalyzed Heck type annulations (*147*), so therefore the ketone was protected as the acetal **7.B6** before the cyclization reaction. The first acetalizing procedure that was tested was a standard procedure using ethanediol and boron trifluoride etherate (*148*). Although, the desired product was obtained with this method the yield was only 25%. Other procedures to promote the acetal formation were subsequently investigated (Table 7.3) and the Noyori method (*149*), which utilizes bis(TMSO)ethane as acetalizing agent and TMS triflate as catalyst, finally gave the product in a satisfactory yield of 82% (Table 7.3, entry 4).

Table 7.3. Results from the different procedures tested for acetal formation of B2

entry	Reaction conditions	Yield
1	BF_3•OEt_2 (12 equiv), ethanediol (26 equiv), DCM, rt, 20h	25%
2	PTSA (0.1 equiv), ethanediol (15 equiv), THF, 50 °C, 6h	14-49%
3	TMSCl (8 equiv), ethanediol (30 equiv), DCM, rt, 48h	0%
4	TMSOTf (0.1 equiv), bis(TMSO)ethane (5 equiv), DCM, -42 °C, 48h	82%

PTSA = *para*-toluene sulfonic acid

The next stage of the synthesis was the cyclization step that had been identified as the most critical step en route to compound B1. The plan was to transform **7.B6** into the fused tricyclic system **7.B7** by using a one-pot tandem sequence of an intramolecular Heck cyclization followed by an intermolecular Suzuki-Miyaura coupling with phenyltrifluoroborate or phenylboronic acid (Scheme 7.9).



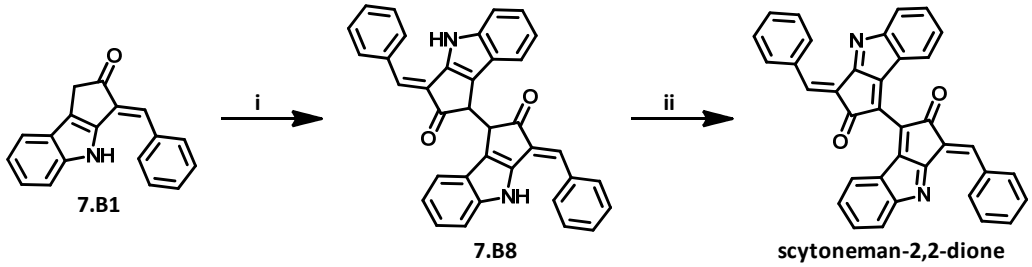
Scheme 7.9. The planed tandem Heck-Suzuki-Miyaura coupling of **7.B6** to obtain **7.B7**. The intramolecular Heck cyclization is believed to take place before the intermolecular Suzuki-Miyaura coupling.

Although, these types of tandem reactions have recently been reported in the literature the employed substrates were rather different (*150, 151*). The first reactions conditions tested were standard Suzuki-Miyaura conditions, when organic trifluoroborates are used as coupling partner (*152*), i.e. palladium acetate as catalyst, triphenylphosphine as ligand, cesiumcarbonate as base, and a mixture of THF and water as solvent. According to the literature similar reaction conditions had been used in intramolecular Heck couplings of alkyne substituted indoles (*153*). Therefore, these reaction conditions were considered promising for the first tandem Heck-Suzuki-Miyaura experiment. The only difference between the tested conditions and those commonly used in Suzuki-Miyaura couplings was the concentration of the substrate, which was decreased substantially to favor the intramolecular Heck cyclization that has to occur before the Suzuki-Miyaura reaction in order for the wanted product to be formed. In contrast to the Pauson-Khand reaction employed in route A, the tandem Heck-Suzuki-Miyaura cyclization gave the desired product in the first attempt (Table 7.4, entry 1). The yield was only 21%, so different conditions were explored (Table 7.4). The highest yield, almost 70%, was obtained with palladium dibenzylideneacetone as catalyst precursor, tri-*tert*-butyl phosphine as ligand, and phenylboronic acid as coupling partner (Table 7.4, entry 4). The last step, before the reduced monomer of scytoneman-2,2-dione was obtained, was deprotection of the acetal and the TMS group, both of which proceeded smoothly using standard conditions.

Table 7.4. Results from the explored conditions in the tandem Heck-Suzuki-Miyaura reaction of 7.B6

entry	Reaction conditions	Yield
1	$Pd(OAc)_2$ (0.05 equiv), PPh_3 (0.1 equiv), $PhBF_3$ (1.1 equiv), Cs_2CO_3 (3 equiv), THF/H_2O (10:1), 70 °C, 19h	21%
2	$Pd(OAc)_2$ (0.1 equiv), PPh_3 (0.2 equiv), $PhBF_3$ (1.1 equiv), Cs_2CO_3 (3 equiv), THF/H_2O (10:1), 70 °C, 7h	32%
3	$Pd(OAc)_2$ (0.1 equiv), $AsPh_3$ (0.3 equiv), $PhBF_3$ (1.1 equiv), Cs_2CO_3 (3 equiv), THF/H_2O (10:1), 70 °C, 6h	31%
4	$Pd_2(dba)_3$ (0.05 equiv), (tBu)$_3$PH[BF]$_4$ (0.2 equiv), $PhB(OH)_2$ (1.5 equiv), Cs_2CO_3 (2.5 equiv), dioxane, 50 °C, 6h	68%

Finally, only the oxidative dimerization remained before the reduced form of scytoneman-2,2-dione (**7.B8**) could be obtained (Scheme 7.10). A large number of successful oxidative couplings of carbonyl enolates can be found in the literature. Different copper and iron salts are often used to facilitate these reactions (*154*), but there are also examples were hypervalent iodine reagents have been used (*155*). Copper (I), copper (II), iron (III) and phenyliodine diacetate (PIDA) were all tested in the oxidative coupling of the monomer **7.B1** (Table 7.5). Neither of the copper salts gave anything but trace amounts of the desired product (Table 7.5, entry 1-2). The hypervalent iodine PIDA, on the other hand, yielded 25 % of the desired coupling product **7.B8** (Table 7.5, entry 3). However, the oxidant that gave the product in highest yield was ferric chloride (Table 7.5, entry 4-5). In the first experiment with ferric chloride, only a slight excess was used of both oxidant and lithium diisopropyl amide (LDA), which gave 40% product (Table 7.5, entry 4). Increase of both ferric chloride and LDA to two equivalents gave a somewhat higher yield (56%, Table 7.5, entry 5). It is also important to note that the product obtained from these reactions was a mixture of several isomers, so therefore no full characterization was performed. Finally an oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) produced scytoneman-2,2-dione (Scheme 7.10) in a total yield of 12% over nine steps.



Scheme 7.10. The synthetic route to scytoneman-2,2-dione from the reduced monomer **7.B1**. Reagents and conditions: (i) See Table 7.5. (ii) DDQ (3 equiv), THF, 0 °C, 45 min, 65%.

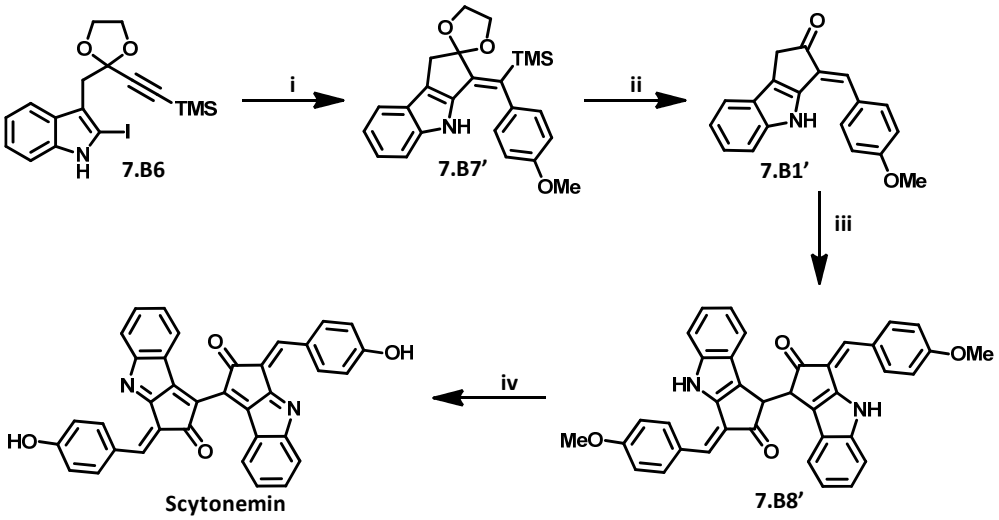
Table 7.5. Results from the different conditions tested in the oxidative coupling of 7.B1

entry	Reaction conditions	Yield
1	CuOTf[a] (2.4 equiv), LDA (2 equiv), THF, -78 °C to rt, 70h	trace[b]
2	$CuCl_2$[a] (1.2 equiv), LDA (1.1 equiv), THF, -78 °C to rt, 0.7h	0%
3	PIDA (0.6 equiv), LDA (1.1 equiv), THF, -78 °C to rt, 2h	25%
4	$FeCl_3$[a] (1.1 equiv), LDA (2.1 equiv), THF, -78 °C to rt, 24h	40%
5	$FeCl_3$[a] (2.2 equiv), LDA (2.1 equiv), THF, -78 °C to rt, 24h	56%

[a] CuOTf was added as an ACN solution. $CuCl_2$ and $FeCl_3$ were added as DMF solutions.
[b] Trace amounts of the product could be identified by HPLC/MS analysis of the reaction mixture.

7.3 Synthetic Route B to Scytonemin

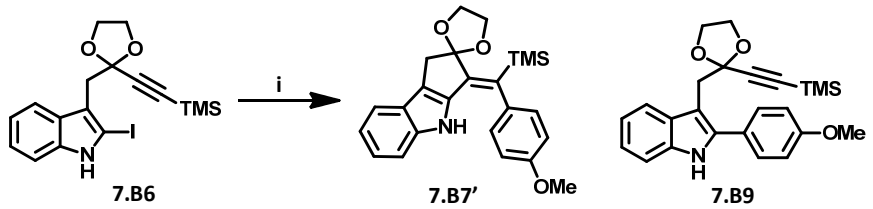
After successful synthesis of the scytonemin derivative scytoneman-2,2-dione, the developed route was tested for synthesis of the natural product itself (Scheme 7.11).



Scheme 7.11. Synthetic route to scytonemin from compound **7.B6**. Reagents and conditions: (i) $Pd_2(dba)_3$ (0.05equiv), $(t\text{Bu})_3\text{PH}[BF]_4$ (0.2 equiv), $PhB(OH)_2$ (1.1 equiv), Cs_2CO_3 (1.8 equiv), dioxane, 50 °C, 6h, 47%. (ii) 1) HCl (5 M, aq), acetone, RT, 30 min, 90%; 2) TBAF (2 equiv), THF, 0 °C, 30 min, 92%. (iii) $FeCl_3$ (2.2 equiv), LDA (2.1 equiv), THF, -78 °C to rt, 24h, 70%. (iv) 1) BBr_3 (4 equiv), DCM, -78 °C to 0 °C, 30 h; 2) DDQ (3 equiv), THF, 0 °C, 45 min, 28%.

The arylgroup, in which the two scytonemin derivatives differ, is introduced in the tandem Heck-Suzuki-Miyaura reaction. The iodinated alkynyl **7.B6** can therefore also be employed in the cyclization reaction to obtain the tricyclic system **7.B7'**, but with a different boronic acid as coupling partner. The oxidative coupling would most likely not succeed with a phenolic substituent. Therefore, a protective group was needed on the phenol. The *para*-methoxphenyl boronic acid was commercially available and was tested in the already developed procedure. The *para*-methoxyboronic acid is more electron-rich than phenylboronic acid and therefore also more reactive in the transmetallation step of the catalytic cycle. The effect of this was that the intermolecular Suzuki-Miyaura coupling competed efficiently with the intramolecular Heck coupling and a mixture of the wanted

product **7.B7'** and the direct Suzuki-Miyaura byproduct **7.B9** (Scheme 7.12) was obtained when the same conditions were used as in the corresponding reaction with phenylboronic acid as coupling partner. To suppress the formation of **7.B9**, the amount of *para*-methoxyboronic acid and base had to be decreased. Unfortunately this also gave a lower yield of the desired cyclized product **7.B7'**.



Scheme 7.12. The products obtained in the first Heck-Suzuki-Miyaura coupling with *para*-methoxyphenylboronic acid. Reagents and conditions: (i) $Pd_2(dba)_3$ (0.05equiv), $(tBu)_3PH[BF]_4$ (0.2 equiv), $PhB(OH)_2$ (1.5 equiv), Cs_2CO_3 (2.5 equiv), dioxane, 50 °C, 6h.

Nevertheless, 47% was at that moment considered a reasonable yield, since the main priority was to obtain the natural product in order to study it. The next step, the deprotection of the acetal and the TMS group, proceed smoothly and the reduced but methyl protected scytonemin monomer **7.B1'** was in hand. The optimal conditions for the oxidative coupling in the route to scytoneman-2,2-dione were also tested on substrate **7.B1'**. In agreement with the literature, the more electron-rich aromatic group conjugated to the enolate in **7.B1'** resulted in a somewhat higher yield compared to when **7.B1** was used as substrate (70% compared to 56%) in the iron mediated oxidative coupling (*151*). The protected phenolic function was then demethylated using standard conditions, which afforded the reduced scytonemin. Finally, oxidation with DDQ successfully resulted in the desired product scytonemin in a total yield of 4% over 10 steps.

7.4 Concluding Discussion

The first attempted synthetic route to the scytonemin derivative scytoneman-2,2-dione was not very successful. At the beginning of the planned route, problems arose when a conjugated ketone should be reduced to a vinylic alcohol. Both sodium borohydride and lithium aluminum hydride were employed as reducing agents, with and without the Lewis acidic metal cerium, but none of the tested conditions gave the desired product. The route was therefore slightly altered and the Pauson-Khand cyclization was first tried on the conjugated ketone, but only starting material was recovered from these attempts. However, this was not really unexpected since alkenes with electron with-drawing groups are generally considered as bad substrates in this reaction (*156*). A substrate that completely lacked the ketone was therefore employed in the Pauson-Khand reaction instead and one of these attempts actually formed what seemed to be trace amounts of the desired product. Unfortunately, this reaction gave a complex mixture of different compounds with a 1,3-diene as one of the major products. Some alterations of the reaction conditions were made in an attempt to increase the amount of the desired product, but neither of these attempts were successful. According to the literature, diene formation is one of the most common outcomes when the Pauson-Khand reaction fails and the reason is usually that β-elimination takes place instead of carbon monoxide insertion (*143*). This mostly happens under thermal conditions and for substrates that either contains a high degree of substitution or polar

groups close to the alkyne or alkene (*143*). However, diene formation has also been observed for substrates that do not seem to be either polarized or highly substituted; for example 1,3-dienes were formed in Pauson-Khand reactions with substituted styrenes and phenyl acetylene (*143*). Furthermore, the formation of dienes is common in all kinds of intramolecular Pauson-Khand reactions of enynes (*157*) (*158*) and rather small changes in the substrate may alter the product outcome completely (*159*). These difficulties in predicting whether a substrate would successfully undergo a Pauson-Khand reaction or not, together with the fact that seven unproductive attempts had already been made, lead to the decision to abandon this synthetic route.

The next route that was explored turned out to be far more successful. The first step that required optimization was the acetal protection of the ketone. The attempted standard conditions for acetal protection gave the desired product but in poor to moderate yields. However, finally the method developed by Tsunoda et al. (*149*), where bis(TMSO)ethane is used as acetalizing agent and TMS triflate as catalyst, resulted in a satisfactory yield. The theoretically challenging tandem Heck-Suzuki-Miyaura cyclization actually gave the desired product on the first attempt of making the scytoneman-2,2-dione precursor and further optimization of this reaction improved the yield from approximately 20 to 70%. The corresponding reaction to obtain the scytonemin precursor was also successful, but the yield was slightly lower. After deprotection of the acetal and silyl protective groups, it was time for the bioinspired oxidative coupling. By screening of a few different oxidative coupling agents, ferric chloride was found to provide the reduced form of scytoneman-2,2-dione in highest yield. Applying these conditions to the oxidative coupling of the methyl protected scytonemin monomer resulted in an even higher yield. The reduced form of the scytonemin-2,2-dione was oxidized and the scytonemin precursor was demethylated and oxidized, which afforded both of the wanted compounds in a total yield of 12 and 4%, respectively.

The first total synthesis of the natural product scytonemin is thereby completed and the desired studies of this interesting compound can now begin. As already mentioned, it would be of interest to study its photochemical and photophysical properties to better understand which structural elements that are important in order to obtain a compound that is both photostable and shows broad absorption of the solar UV radiation. In order to explore this, structure activity studies of scytonemin derivatives with *para* substituents with different electronic properties are currently being undertaken. Also, comparative studies of the monomers and dimers are of interest to understand why this compound exists as a dimer. Another problem with scytonemin, in the context of a UV-filter suitable for incorporation in commercial sunscreens, is its poor solubility. Therefore, syntheses of derivatives with substituents, other than aryl groups, that are more lipophilic are also planned for the future. These studies will provide more knowledge regarding the construction of a photostable UV-filter suitable for commercial skin products. In parallel, it is also important to evaluate the toxicity and allergenicity of these compounds to ensure that a UV-filter without unwanted side effects is proposed.

8 GENERAL DISCUSSION

Photocontact allergy is considered to be an uncommon disorder (*5*). However, not many clinics worldwide perform photopatch testing and therefore this problem may be underestimated. If one considers reports from those clinics that do perform photopatch testing (*37, 38, 53, 56, 113*), in my opinion the incidence of photocontact allergy do not appear to be uncommon, especially not photocontact allergy to ketoprofen. It is unfortunate that not more clinics perform photopatch testing for two reasons. Firstly, some patients will not know which compound they are allergic to, and therefore cannot avoid it. Secondly, valuable information could be retrieved that would facilitate the ongoing regulatory work to decrease the exposure of compounds that induce contact allergy.

It has been known for more than 15 years that the dibenzoylmethanes degrade when irradiated (*45, 46*). Despite that, 4-*tert*-butyl-4'-methoxy dibenzoylmethane is still the most commonly used chemical UVA-filter in commercial sunscreens. It appears as if the industry is more focused on developing substances that will stabilize 4-*tert*-butyl-4'-methoxy dibenzoylmethane instead of trying to develop a new UVA-filter that does not degrade when irradiated. Furthermore, when reports emerged in the eighties and early nineties concerning photocontact allergy to 4-isopropyldibenzoylmethane, which was the dibenzoylmethane used in commercial sunscreens at that time, the industry simply replaced it with the very similar 4-*tert*-butyl-4'-methoxy dibenzoylmethane. In paper I, we showed that photocontact allergy to the dibenzoylmethanes is probably caused by arylglyoxals produced when the dibenzoylmethanes photodegrade. The arylglyoxals were shown to be strong skin sensitizers based on the murine LLNA and thereby very low concentrations are enough to cause an allergic reaction. We also showed that *para* substituents with different electronic effects have no impact on the sensitizing capacity of the arylglyoxals. It therefore seems highly unlikely that it would be possible to construct a *para* substituted dibenzoylmethane without photoallergenic properties. It is also interesting that the benzils that are formed when the dibenzoylmethanes degrade posses cytotoxic properties. The clinical implication of these results remains to be investigated.

The use of octocrylene has increased substantially in the last few years and today octocrylene is one of the most commonly used chemical UV-filters. The frequent incorporation of octocrylene in sunscreens developed for children appears to already have sensitized a large number of children (*53*). In paper II we established that octocrylene is a moderate sensitizer in the LLNA and that it reacts with the amino acid lysine via a retro-aldol condensation reaction. This reactivity toward amines is probably the reason for octocrylene's contact allergenic ability. Astonishingly, octocrylene is allowed by both the EU and the FDA in as high concentrations as 10% in sunscreens and cosmetics. Today there are not many compounds that are known to be moderate or strong sensitizers in themselves (no activation either metabolically or by air oxidation is needed) that are allowed for use in cosmetic products.

The preservative methylisothiazolinone (MI) is another moderate allergen, according to the LLNA (*160*), that is approved for use in cosmetics and toiletries (*161*). However, the maximum allowed concentration in the EU is 0.01% (*161*) and it is still sensitizes a significant number of people (*162-165*). Furthermore, one should keep in mind that a sunscreen is a stay-on product and is often repeatedly applied. Therefore, the actual dose that one could

be subjected to may be very high. Considering the fact that octocrylene is incorporated in most commercial sunscreens, there is an imminent risk that a large number of people will become sensitized to it.

In paper III we showed that UV radiation both increases the reactivity of octocrylene and changes the reaction pathway. In the absence of UV radiation the mechanism by which the model nucleophile benzylamine reacts with octocrylene is a retro-aldol condensation, whereas an amide formation occurs when benzylamine reacts with octocrylene in presence of UV radiation. In the context of contact and photocontact allergy this suggests that octocrylene has the ability to form immunogenic complexes via two different pathways. This is important when it comes to testing of patients with a suspected allergy to octocrylene, since patients sensitized to the immunogenic complex formed in presence of UV radiation will not display a reaction without irradiation. It is therefore necessary to do both patch and photopatch testing to rule out allergy to octocrylene. Furthermore, this change in reactivity of octocrylene is also of great importance when it comes to understanding the possible mechanisms behind photocontact allergy, of which surprisingly little is known.

In the clinical study performed in paper II, as well as in a few other clinical studies (*53, 56*), it has been noted that photocontact allergy to ketoprofen appears to also lead to photocontact allergy to octocrylene. Furthermore, photocontact allergy to ketoprofen has been associated with other apparent photocross allergies to a large number of structurally diverse compounds. In paper IV we therefore set out to study ketoprofen's interaction with amino acid analogs when irradiated, in order to find the reason for these puzzling clinical observations. Interestingly, ketoprofen substantially enhanced the formation rate of an adduct between the amino acid analogs of tryptophan and lysine. The first step of this adduct formation is believed to be a reaction of the tryptophan analog with singlet molecular oxygen. We therefore believe that ketoprofen's ability to promote the formation of this adduct is due to its capacity to generates singlet molecular oxygen when irradiated in presence of air. These results imply that ketoprofen is not part of the immunogenic complex itself; instead it enhances a reaction between two amino acid residues. The formation of an immunogenic complex that does not contain the allergen itself explains the wide variety of molecular structure of other allergens to which ketoprofen allergic patients also react to in presence of light. In other words, all compounds that are able to generate singlet molecular oxygen also have the capacity to promote the formation of the same immunogenic complex. This is very unfortunate for the patients that have been photosensitized to this particular complex, since it will be very hard for them to avoid all of these compounds and as a consequence they will probably often suffer from allergic reactions after solar exposure. Whether the tryptophan and lysine residues that form the immunogenic complex are situated within the same protein or if it is a reaction between two different proteins is not known. It would therefore be interesting to conduct the corresponding studies with a peptide or a small protein instead of the amino acid analogs. To explore the possibility that octocrylene may cause allergic reactions via a third pathway leading to different immunogenic complexes than the other two routes, octocrylene's capacity to promote the formation of this Trp-Lys adduct is currently being investigated.

Although, sunscreens have been around for over eighty years, surprisingly little is known about what effect the photochemical and photophysical properties of the active ingredients have on biological material. In addition, the recommended way to protect oneself from harmful solar radiation is by use of sunscreens. A large proportion of the public is therefore

frequently exposed to these UV-filters. One important unwanted side-effect of the use of these compounds is of course the risk of developing contact and photocontact allergy, which has been discussed extensively in this thesis. However, another important danger with this constant use of sunscreens containing photolabile UV-filters is the false security that it may imply. The actual protection, especially in UVA range, may not be as good as people believe, which may lead to people spending longer time in the sun than they would had done without a sunscreen. This could in fact lead to an increased risk of developing skin cancer. It is therefore important to develop a photostable UV-filter, without unwanted side-effects like contact and photocontact allergy, which can be used in commercial products. However, to do this we first need to understand how a molecule should be constructed in order for it to be photostable. For that reason, in paper V, a synthetic route to the natural UV-filter scytonemin was developed. Scytonemin protects cyanobacteria that live in habitats were the solar radiation is very intense and can cause cellular damage. Paper V is the first published total synthesis of this natural occurring dimeric alkaloid. The planned structure-activity studies of this compound and derivatives thereof will most likely provide a deeper knowledge about which structural elements are important in the construction of a photostable compound. Hopefully, this knowledge can be used to develop a better UV-filter for commercial use than the ones that are on the market today.

Four compounds that all absorb UV radiation, but with large differences in photostability, have been studied within this work. The NSAID ketoprofen is at one end of the photostability scale, displaying extremely photolabile properties. Considering that this is the only one of the studied compounds that is not intended as a UV-filter this may not be very surprising. However, it is highly inappropriate to use such a photounstable compound in topical preparations, especially since it also possesses remarkable photosensitizing abilities. The dibenzoylmethanes can be found in the middle of the photostability scale. They are considerably more stable than ketoprofen, but as they are intended to be UV-filters their photostability is not very impressive. Substantial photodegradation of the dibenzoylmethanes was noted, which leads both to loss in the protective ability of the sunscreen and to the formation of compounds with both allergenic and cytotoxic properties. Octocrylene, on the other hand, displayed excellent photostability properties when irradiated in cyclohexane and in ethanol only minor depletion of octocrylene could be seen. However, the depletion that was seen was due to its reaction with ethanol, which can be seen as an analog for amino acids with hydroxyl functions, and in presence of different amino acid analogs octocrylene also reacted with the lysine analog (benzylamine). This proves that photostability is not the only property that needs to be accounted for when developing UV-filters. It is also important to study the photochemical properties of the UV-filter on a molecular level to decrease the risk of launching a compound with the ability to induce contact and photocontact allergy. Unfortunately, we have not yet tested the photostability of the natural UV-filter scytonemin in our photoreactor. Therefore, it is not possible to compare its photostability to the other compounds studied in this work. However, previous photostability studies of scytonemin (*14, 166, 167*) together with the proposed theory that it was developed to protect cyanobacteria from UV radiation during their early evolutionary stage when there was no stratospheric ozone layer (*167*) strongly indicates that scytonemin possesses extraordinary photoprotective abilities. In addition to further studies of scytonemin's stability when irradiated, studies of its allergenic and cytotoxic properties are also needed before we know if scytonemin or any of its derivatives may be suitable for incorporation in a commercial sunscreen.

In conclusion, the studies in this thesis have contributed to the overall understanding of the chemistry behind photocontact allergy. It has been demonstrated that different compounds have the possibility to induce photocontact allergy via completely different mechanistic pathways:

i. The photoallergenic ability of the dibenzoylmethanes is most likely due to fragmentation which results in a photoproduct (arylglyoxal) that serves as a hapten.
ii. Octocrylene may have the ability to induce photocontact allergy, via a reaction of the excited state molecule with amines and alcohols.
iii. Ketoprofen probably induces photocontact allergy via energy transfer where singlet molecular oxygen is generated, which subsequently modifies a protein in such a way that the immune system will think of it as non-self.

Octocrylene appears to also have the ability to induce contact allergy, via a ground state reaction with amines. Furthermore, according to clinical reports it seems as if octocrylene has the ability to elicit photoallergic reactions in patients with photocontact allergy to ketoprofen, which indicates that octocrylene is also able to generate singlet molecular oxygen via energy transfer. However, further studies are needed to confirm this hypothesis. In addition, the first total synthesis to the natural UV-filter scytonemin has been developed and studies of its photochemical and photophysical properties will provide deeper knowledge regarding construction of a photostable UV-filter.

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10 REFERENCES

(1) Mang, R., Stege, H., and Krutmann, J. (2006) Mechanisms of phototoxic and photoallergic reactions, In *Contact Dermatitis* (Frosch, P. J., Menné, T., and Lepoittevin, J.-P., Eds.) pp 97-104, Springer-Verlag, Berlin Heidelberg.

(2) Norval, M., and Wulf, H. C. (2009) Does chronic sunscreen use reduce vitamin D production to insufficient levels? *Br J Dermatol*, *161*, 732-736.

(3) Wang, S. Q., Balagula, Y., and Osterwalder, U. (2010) Photoprotection: a Review of the Current and Future Technologies. *Dermatologic Therapy*, *23*, 31-47.

(4) Maier, T., and Korting, H. C. (2005) Sunscreens - Which and what for? *Skin Pharmacol Physiol*, *18*, 253-262.

(5) Lautenschlager, S., Wulf, H. C., and Pittelkow, M. R. (2007) Photoprotection. *Lancet*, *370*, 528-537.

(6) Barysch, M. J., Hofbauer, G. F., and Dummer, R. (2010) Vitamin D, ultraviolet exposure, and skin cancer in the elderly. *Gerontology*, *56*, 410-413.

(7) Commission recommendation of the EC, E. (2006) COMISSION RECOMMENDATION of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto. *Official Journal of the European Union*, *265*, 39-43.

(8) Council Directive of the EC, E. U. (2008) Annex VII: List of UV filters which cosmetic products may contain, In *76/768/EEC* pp 121-126.

(9) Department of Health and Human Services, F. U. (1999) Sunscreen Drug Products For Over-The-Counter Human Use: Final Monograph. *Federal Register*, *64*, 27666-27693.

(10) Westerdahl, J., Ingvar, C., Masback, A., and Olsson, H. (2000) Sunscreen use and malignant melanoma. *International Journal of Cancer*, *87*, 145-150.

(11) Burnett, M. E., and Wang, S. Q. (2011) Current sunscreen controversies: a critical review. *Photodermatology Photoimmunology & Photomedicine*, *27*, 58-67.

(12) Bryden, A. M., Moseley, H., Ibbotson, S. H., Chowdhury, M. M. U., Beck, M. H., Bourke, J., English, J., Farr, P., Foulds, I. S., Gawkrodger, D. J., George, S., Orton, D. I., Shaw, S., McFadden, J., Norris, P., Podmore, P., Powell, S., Rhodes, L. E., Sansom, J., Wilkinson, M., van Weelden, H., and Ferguson, J. (2006) Photopatch testing of 1155 patients: results of the UK multicentre photopatch study group. *British Journal of Dermatology*, *155*, 737-747.

(13) Victor, F. C., Cohen, D. E., and Soter, N. A. (2010) A 20-year analysis of previous and emerging allergens that elicit photoallergic contact dermatitis. *Journal of the American Academy of Dermatology*, *62*, 605-610.

(14) Garciapichel, F., and Castenholz, R. W. (1991) CHARACTERIZATION AND BIOLOGICAL IMPLICATIONS OF SCYTONEMIN, A CYANOBACTERIAL SHEATH PIGMENT. *Journal of Phycology*, *27*, 395-409.

(15) Proteau, P. J., Gerwick, W. H., Garciapichel, F., and Castenholz, R. (1993) THE STRUCTURE OF SCYTONEMIN, AN ULTRAVIOLET SUNSCREEN PIGMENT FROM THE SHEATHS OF CYANOBACTERIA. *Experientia*, *49*, 825-829.

(16) Fleming, E. D., and Castenholz, R. W. (2007) Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. *Environmental Microbiology*, *9*, 1448-1455.

(17) Stevenson, C. S., Capper, E. A., Roshak, A. K., Marquez, B., Eichman, C., Jackson, J. R., Mattern, M., Gerwick, W. H., Jacobs, R. S., and Marshall, L. A. (2002) The identification and characterization of the marine natural product scytonemin as a novel antiproliferative pharmacophore. *Journal of Pharmacology and Experimental Therapeutics*, *303*, 858-866.

(18) Stevenson, C. S., Capper, E. A., Roshak, A. K., Marquez, B., Grace, K., Gerwick, W. H., Jacobs, R. S., and Marshall, L. A. (2002) Scytonemin - a marine natural product inhibitor of kinases key in hyperproliferative inflammatory diseases. *Inflammation Research*, *51*, 112-114.
(19) Takamatsu, S., Hodges, T. W., Rajbhandari, I., Gerwick, W. H., Hamann, M. T., and Nagle, D. G. (2003) Marine natural products as novel antioxidant prototypes. *Journal of Natural Products*, *66*, 605-608.
(20) Nielsen, N. H., Linneberg, A., Menne, T., Madsen, F., Frolund, L., Dirksen, A., and Jorgensen, T. (2001) Allergic contact sensitization in an adult Danish population: Two cross-sectional surveys eight years apart (The Copenhagen Allergy Study). *Acta Dermato-Venereologica*, *81*, 31-34.
(21) Thyssen, J. P., Linneberg, A., Menne, T., and Johansen, J. D. (2007) The epidemiology of contact allergy in the general population - prevalence and main findings. *Contact Dermatitis*, *57*, 287-299.
(22) Kimber, I., Basketter, D. A., Gerberick, G. F., and Dearman, R. J. (2002) Allergic contact dermatitis. *International Immunopharmacology*, *2*, 201-211.
(23) Thyssen, J. P., and Menne, T. (2010) Metal Allergy-A Review on Exposures, Penetration, Genetics, Prevalence, and Clinical Implications. *Chemical Research in Toxicology*, *23*, 309-318.
(24) de Groot, A. C., and Maibach, H. I. (2010) Frequency of sensitization to common allergens: comparison between Europe and the USA. *Contact Dermatitis*, *62*, 325-329.
(25) Karlberg, A. T., Bergstrom, M. A., Borje, A., Luthman, K., and Nilsson, J. L. G. (2008) Allergic contact dermatitis-formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol*, *21*, 53-69.
(26) Rustemeyer, T., van Hoogstraten, I. M. W., von Blomberg, B. M. E., and Scheper, R. J. (2006) Mechanisms in allergic contact dermatitis, In *Contact Dermatitis* (Frosch, P. J., Menné, T., and Lepoittevin, J.-P., Eds.) pp 11-43, Berlin Heidelberg, Springer-Verlag.
(27) Divkovic, M., Pease, C. K., Gerberick, G. F., and Basketter, D. A. (2005) Hapten-protein binding: from theory to practical application in the in vitro prediction of skin sensitization. *Contact Dermatitis*, *53*, 189-200.
(28) Schmidt, R. J., Khan, L., and Chung, L. Y. (1990) Are free radicals and not quinones the haptenic species derived from urushiols and other contact allergenic mono- and dihydric alkylbenzenes? The significance of NADH, glutathione, and redox cycling in the skin. *Arch Dermatol Res*, *282*, 56-64.
(29) Xia, Z., Miyakoshi, T., and Yoshida, T. (2004) Lipoxygenase-catalyzed polymerization of phenolic lipids suggests a new mechanism for allergic contact dermatitis induced by urushiol and its analogs. *Biochem Biophys Res Commun*, *315*, 704-709.
(30) Johansson, S., Gimenez-Arnau, E., Grotli, M., Karlberg, A. T., and Borje, A. (2008) Carbon- and oxygen-centered radicals are equally important haptens of allylic hydroperoxides in allergic contact dermatitis. *Chemical Research in Toxicology*, *21*, 1536-1547.
(31) Johansson, S., Redeby, T., Altamore, T. M., Nilsson, U., and Borje, A. (2009) Mechanistic Proposal for the Formation of Specific Immunogenic Complexes via a Radical Pathway: A Key Step in Allergic Contact Dermatitis to Olefinic Hydroperoxides. *Chemical Research in Toxicology*, *22*, 1774-1781.
(32) Lepoittevin, J.-P., and Goossens, A. (1998) Molecular Basis for the Recognition of Haptens by T Lymphocytes, In *Allergic Contact Dermatitis* (Lepoittevin, J.-P.,

Basketter, D., Goossens, A., and Karlberg, A. T., Eds.) pp 112-128, Springer-Verlag, Berlin Heidelberg New York.

(33) Christensson, J. B., Johansson, S., Hagvall, L., Jonsson, C., Borje, A., and Karlberg, A. T. (2008) Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis*, *59*, 344-352.

(34) Christensson, J. B., Matura, M., Backtorp, C., Borje, A., Nilsson, J. L. G., and Karlberg, A. T. (2006) Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis*, *55*, 230-237.

(35) Karlberg, A. T., Nilsson, A. M., Gafvert, E., Salvador, L., Luthman, K., Bruze, M., Gruvberger, B., and Nilsson, J. L. G. (2001) Mechanism of the antigen formation of carvone and related alpha,beta-unsaturated ketones. *Contact Dermatitis*, *44*, 347-356.

(36) Wayne, C. E., and Wayne, R. P. (1996) *Photochemistry*. Oxford University Press.

(37) Cardoso, J. C., Canelas, M. M., Goncalo, M., and Figueiredo, A. (2009) Photopatch testing with an extended series of photoallergens: a 5-year study. *Contact Dermatitis*, *60*, 325-329.

(38) Pigatto, P. D., Guzzi, G., Schena, D., Guarrera, M., Foti, C., Francalanci, S., Cristaudo, A., Ayala, F., and Vincenzi, C. (2008) Photopatch tests: an Italian multicentre study from 2004 to 2006. *Contact Dermatitis*, *59*, 103-108.

(39) Goossens, A. (2004) Photoallergic contact dermatitis. *Photodermatology, Photoimmunology and Photomedicine*, *20*, 121-125.

(40) Bakkum, R., and Heule, F. (2002) Results of photopatch testing in Rotterdam during a 10-year period. *British Journal of Dermatology*, *146*, 275-279.

(41) Darvay, A., White, I. R., Rycroft, R. J. G., Jones, A. B., Hawk, J. L., and McFadden, J. P. (2001) Photoallergic contact dermatitis is uncommon. *British Journal of Dermatology*, *145*, 597-601.

(42) Damiani, E., Rosati, L., Castagna, R., Carloni, P., and Greci, L. (2006) Changes in ultraviolet absorbance and hence in protective efficacy against lipid peroxidation of organic sunscreens after UVA irradiation. *Photochem Photobiol B Biol*, *82*, 204-213.

(43) Scalia, S., and Mezzena, M. (2009) Incorporation in Lipid Microparticles of the UVA Filter, Butyl Methoxydibenzoylmethane Combined with the UVB Filter, Octocrylene: Effect on Photostability. *AAPS PharmSciTech*, *10*, 384-390.

(44) Herzog, B., Wehrle, M., and Quass, K. (2009) Photostability of UV Absorber Systems in Sunscreens. *Photochem Photobiol*, *85*, 869-878.

(45) Schwack, W., and Rudolph, T. (1995) Photochemistry of dibenzoyl methane UVA filters Part 1. *J. Photochem. Photobiol. B: Biol.*, *28*, 229-234.

(46) Roscher, N. M., Lindemann, M. K. O., Kong, S. B., Cho, C. G., and Jiang, P. (1994) Photodecomposition of several compounds commonly used as sunscreen agents. *Journal of Photochemistry and Photobiology a-Chemistry*, *80*, 417-421.

(47) Tarras-Wahlberg, N., Stenhagen, G., Larko, O., Rosen, A., Wennberg, A. M., and Wennerstrom, O. (1999) Changes in ultraviolet absorption of sunscreens after ultraviolet irradiation. *Journal of Investigative Dermatology*, *113*, 547-553.

(48) Duracher, L., Blasco, L., Jaoued, A. A., Vian, L., and Marti-Mestres, G. (2009) Irradiation of Skin and Contrasting Effects on Absorption of Hydrophilic and Lipophilic Compounds. *Photochem Photobiol*, *85*, 1459-1467.

(49) Gaspar, L. R., and Campos, P. (2006) Evaluation of the photostability of different UV filter combinations in a sunscreen. *International Journal of Pharmaceutics*, *307*, 123-128.

(50) Bonda, C. A. (2005) Photostabilization of a sunscreen composition with a combination of an α-cyano-β, β-diphenylacrylate compound and a dialkyl naphithalate, CPH Innovations Corporation, United States.

(51) Deflandre, A., Dubois, M., Forestier, S., and Richard, H. (1991) Light-resist ant filtering cosmetic composition containing a UV_A filter and an alkyl beta, beta -diphenylacrylate or an alpha-cyano-beta, beta -diphenylacrylate, Watermark patent and trademark attorneys, France.
(52) Delplace, D., and Blondeel, A. (2006) Octocrylene: really non-allergenic? *Contact Dermatitis*, *54*, 295-295.
(53) Avenel-Audran, M., Dutartre, H., Goossens, A., Jeanmougin, M., Comte, C., Bernier, C., Benkalfate, L., Michel, M., Ferrier-Lebouedec, M. C., Vigan, M., Bourrain, J. L., Outtas, O., Peyron, J. L., and Martin, L. (2010) Octocrylene, an Emerging Photoallergen. *Arch Dermatol*, *146*, 753-757.
(54) Carrotte-Lefebvre, I., Bonnevalle, A., Segard, M., Delaporte, E., and Thomas, P. (2003) Contact allergy to octocrylene - First 2 cases. *Contact Dermatitis*, *48*, 46-47.
(55) Madan, V., and Beck, M. H. (2005) Contact allergy to octocrylene in sunscreen with recurrence from passive transfer of a cosmetic. *Contact Dermatitis*, *53*, 241-242.
(56) Devleeschouwer, V., Roelandts, R., Garmyn, M., and Goossens, A. (2008) Allergic and photoallergic contact dermatitis from ketoprofen: results of (photo) patch testing and follow-up of 42 patients. *Contact Dermatitis*, *58*, 159-166.
(57) Lechat, P., and Slanar, O. (2010) Questions and answers on the review of the marketing authorisations for topical formulations of ketoprofen - Outcome of a procedure under Atricle 107 of Directive 2001/83/EC, (European Medicines Agency, E. U., Ed.).
(58) Haroutiunian, S., Drennan, D. A., and Lipman, A. G. (2010) Topical NSAID therapy for musculoskeletal pain. *Pain Med*, *11*, 535-549.
(59) Brewer, A. R., McCarberg, B., and Argoff, C. E. (2010) Update on the use of topical NSAIDs for the treatment of soft tissue and musculoskeletal pain: a review of recent data and current treatment options. *Phys Sportsmed*, *38*, 62-70.
(60) Valsecchi, R., Falgheri, G., and Cainelli, T. (1983) Contact dermatitis from ketoprofen. *Contact Dermatitis*, *9*, 163-164.
(61) Angelini, G., and Vena, G. A. (1983) Contact allergy to ketoprofen. *Contact Dermatitis*, *9*, 234.
(62) Alomar, A. (1985) Ketoprofen photodermatitis. *Contact Dermatitis*, *12*, 112-113.
(63) Albes, B., Marguery, M. C., Schwarze, H. P., Journe, F., Loche, F., and Bazex, J. (2000) Prolonged photosensitivity following contact photoallergy to ketoprofen. *Dermatology*, *201*, 171-174.
(64) Pigatto, P., Bigardi, A., Legori, A., Valsecchi, R., and Picardo, M. (1996) Cross-reactions in patch testing and photopatch testing with ketoprofen, thiaprophenic acid, and cinnamic aldehyde. *Am J Contact Dermat*, *7*, 220-223.
(65) Le Coz, C. J., Bottlaender, A., Scrivener, J. N., Santinelli, F., Cribier, B. J., Heid, E., and Grosshans, E. M. (1998) Photocontact dermatitis from ketoprofen and tiaprofenic acid: cross-reactivity study in 12 consecutive patients. *Contact Dermatitis*, *38*, 245-252.
(66) Durbize, E., Vigan, M., Puzenat, E., Girardin, P., Adessi, B., Desprez, P. H., Humbert, P. H., Laurent, R., and Aubin, F. (2003) Spectrum of cross-photosensitization in 18 consecutive patients with contact photoallergy to ketoprofen: associated photoallergies to non-benzophenone-containing molecules. *Contact Dermatitis*, *48*, 144-149.
(67) Wahlberg, J. E., and Lindberg, M. (2006) Patch Testing, In *Contact Dermatitis* (Frosch, P. J., Menné, T., and Lepoittevin, J.-P., Eds.) pp 365-390, Springer, Berlin Heidelberg New York.
(68) OECD. (2002) OECD Guideline for Testing of Chemicals. Skin Sensitisation: Local Lymph Node Assay. *OECD Guideline*, *429*.

(69) Gerberick, G. F., Ryan, C. A., Dearman, R. J., and Kimber, I. (2007) Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. *Methods*, *41*, 54-60.
(70) Basketter, D., Lea, L., Dickens, A., Briggs, D., Pate, I., Dearman, R., and Kimber, I. (1999) A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxicol*, *19*, 261-266.
(71) Kimber, I., Basketter, D., Butler, M., Gamer, A., Garrigue, J. L., Gerberick, F., Newsome, C., Steiling, W., and Vohr, H. W. (2003) Classification of contact allergens according to potency: Proposals. *Food Chem Toxicol*, *41*, 1799-1809.
(72) Barltrop, J. A., Owen, T. C., Cory, A. H., and Cory, J. G. (1991) 5-(3-Carboxymethoxyphenyl)-2-(4,5-Dimethylthiazolyl)-3-(4-Sulfophenyl)Tetrazolium, Inner Salt (Mts) and Related Analogs of 3-(4,5-Dimethylthiazolyl)-2,5-Diphenyltetrazolium Bromide (Mtt) Reducing to Purple Water-Soluble Formazans as Cell-Viability Indicators. *Bioorg Med Chem Lett*, *1*, 611-&.
(73) Cory, A. H., Owen, T. C., Barltrop, J. A., and Cory, J. G. (1991) Use of an Aqueous Soluble Tetrazolium Formazan Assay for Cell-Growth Assays in Culture. *Cancer Commun*, *3*, 207-212.
(74) Riss, T. L., and Moravec, R. A. (1992) Comparison of Mtt, Xtt, and a Novel Tetrazolium Compound Mts for Invitro Proliferation and Chemosensitivity Assays. *Mol Biol Cell*, *3*, A184-A184.
(75) Bosse, D., and Meijere, A. D. (1974) Photoisomerization of tricyclo[5.2.1.04,10]deca-2,5,8-triene (triquinacene). *Angewandte Chemie-International Edition in English*, *13*, 663-664.
(76) Hu, A., and Lin, W. (2005) Ru-Catalyzed Asymmetric Hydrogenation of α-Phthalimide Ketones and 1,3-Diaryl Diketones Using 4,4'-Substituted BINAPs. *Org. Lett.*, *7*, 455-458.
(77) Floyd, M. B., Du, M. T., Fabio, P. F., Jacob, L. A., and Johnson, B. D. (1985) The Oxidation of Acetophenones to Arylglyoxals with Aqueous Hydrobromic Acid in Dimethyl Sulfoxide. *Journal of Organic Chemistry*, *50*, 5022-5027.
(78) Luo, H. K., Yang, H. Y., Jie, T. X., Chiew, O. S., Schumann, H., Khim, L. B., and Lim, C. (2007) Water-tolerant enantioselective carbonyl-ene reactions with palladium(II) and platinum(II) Lewis acid catalysts bearing BINAP. *Journal of Molecular Catalysis a-Chemical*, *261*, 112-119.
(79) Zuliani, V., Cocconcelli, G., Fantini, M., Ghiron, C., and Rivara, M. (2007) A practical synthesis of 2,4(5)-diarylimidazoles from simple building blocks. *Journal of Organic Chemistry*, *72*, 4551-4553.
(80) Becker, H. D., and Russell, G. A. (1963) Structure of hemihydrates of phenylglyoxals. *Journal of Organic Chemistry*, *28*, 1895-&.
(81) Howe, R., McLoughl.Bj, Rao, B. S., Smith, L. H., and Chodneka.Ms. (1969) Beta-adrenergic blocking agents .4. variation of 2-naphthyl group of pronethalol [2-isopropylamino-1-(2-naphthyl)ethonol]. *Journal of Medicinal Chemistry*, *12*, 452-&.
(82) Kua, J., Hanley, S. W., and De Haan, D. O. (2008) Thermodynamics and kinetics of glyoxal dimer formation: A computational study. *Journal of Physical Chemistry A*, *112*, 66-72.
(83) Feng, X. L., Pisula, W., Takase, M., Dou, X., Enkelmann, V., Wagner, M., Ding, N., and Mullen, K. (2008) Synthesis, helical organization, and fibrous formation of C-3 symmetric methoxy-substituted discotic hexa-peri-hexabenzocoronene. *Chemistry of Materials*, *20*, 2872-2874.
(84) Mousset, C., Provot, O., Hamze, A., Bignon, J., Brion, J. D., and Alami, M. (2008) DMSO - PdI2 as a powerful oxidizing couple of alkynes into benzils: one-pot

synthesis of nitrogen-containing five- or six-membered heterocycles. *Tetrahedron*, *64*, 4287-4294.

(85) Faghihi, K., Zamani, K., Mirsamie, A., and Sangi, M. R. (2003) Microwave-assisted rapid synthesis of novel optically active poly(amide-imide)s containing hydantoins and thiohydantoins in main chain. *European Polymer Journal*, *39*, 247-254.

(86) Khurana, J. M., Chauhan, S., and Bansal, G. (2004) Facile hydrolysis of esters with KOH-methanol at ambient temperature. *Monatshefte Fur Chemie*, *135*, 83-87.

(87) Ashby, J., Basketter, D. A., Paton, D., and Kimber, I. (1995) Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology*, *103*, 177-194.

(88) Ryan, C. A., Gerberick, G. F., Cruse, L. W., Basketter, D., Lea, L., Blaikie, L., Dearman, R. J., Warbrick, E. V., and Kimber, I. (2000) Activity of human contact allergens in the murine local lymph node assay. *Contact Dermatitis*, *43*, 90-102.

(89) Patlewicz, G., Basketter, D. A., Smith, C. K., Hotchkiss, S. A. M., and Roberts, D. W. (2001) Skin-sensitization structure-activity relationships for aldehydes. *Contact Dermatitis*, *44*, 331-336.

(90) Anderson, S. E., Wells, J. R., Fedorowicz, A., Butterworth, L. F., Meade, B. J., and Munson, A. E. (2007) Evaluation of the contact respiratory sesitization potential of volatile organic compounds generated by simulated indoor air chemistry. *Toxicological Sciences*, *97*, 355-363.

(91) Roberts, D. W., York, M., and Basketter, D. A. (1999) Structure-activity relationships in the murine local lymph node assay for skin sensitization: alpha,beta-diketones. *Contact Dermatitis*, *41*, 14-17.

(92) Atsumi, T., Iwakura, I., Fujisawa, S., and Ueha, T. (2001) The production of reactive oxygen species by irradiated camphorquinone-related photosensitizers and their effect on cytotoxicity. *Archives of Oral Biology*, *46*, 391-401.

(93) Mousset, C., Giraud, A., Provot, O., Hamze, A., Bignon, J., Liu, J. M., Thoret, S., Dubois, J., Brion, J. D., and Alami, M. (2008) Synthesis and antitumor activity of benzils related to combretastatin A-4. *Bioorganic and Medicinal Chemistry Letters*, *18*, 3266-3271.

(94) Takahashi, K. (1968) The Reaction of Phenylglyoxal with Arginine Residues in Proteins. *Journal of Biological Chemistry*, *243*, 6171-6179.

(95) Saraiva, M. A., Borges, C. M., and Florencio, M. H. (2006) Non-enzymatic model glycation reactions - a comprehensive study of the reactivity of a modified arginine with aldehydic and diketonic dicarbonyl compounds by electrospray mass spectrometry. *Journal of Mass Spectrometry*, *41*, 755-770.

(96) Kligman, A. M. (1966) Identification of contact allergens by human assay .2. factors influencing induction and measurement of allergic contact dermatitis. *Journal of Investigative Dermatology*, *47*, 375-&.

(97) Friedmann, P. S. (2007) The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *British Journal of Dermatology*, *157*, 1093-1102.

(98) Vigan, M. (2002) REVIDAL-GERDA: organisation and collaboration with pharmacovigilance. *Therapie*, *57*, 263-264.

(99) Ahlfors, S. R., Sterner, O., and Hansson, C. (2003) Reactivity of contact allergenic haptens to amino acid residues in a model carrier peptide, and characterization of formed peptide-hapten adducts. *Skin Pharmacol Appl Skin Physiol*, *16*, 59-68.

(100) Nilsson, A. M., Gafvert, E., Salvador, L., Luthman, K., Bruze, M., Gruvberger, B., Nilsson, J. L. G., and Karlberg, A. T. (2001) Mechanism of the antigen formation of carvone and related alpha,beta-unsaturated ketones. *Contact Dermatitis*, *44*, 347-356.

(101) Schultz, T. W., Yarbrough, J. W., and Johnson, E. L. (2005) Structure-activity relationships for reactivity of carbonyl-containing compounds with glutathione. *SAR QSAR Environ Res*, *16*, 313-322.
(102) Schultz, T. W., Yarbrough, J. W., Hunter, R. S., and Aptula, A. O. (2007) Verification of the structural alerts for Michael acceptors. *Chem Res Toxicol*, *20*, 1359-1363.
(103) Estrada, E., Patlewicz, G., and Gutierrez, Y. (2004) From knowledge generation to knowledge archive. a general strategy using TOPS-MODE with DEREK to formulate new alerts for skin Sensitization. *J Chem Inf Comput Sci*, *44*, 688-698.
(104) Cai, J., Bhatnagar, A., and Pierce, W. M. (2009) Protein Modification by Acrolein: Formation and Stability of Cysteine Adducts. *Chem Res Toxicol*, *22*, 708-716.
(105) Enoch, S. J., Cronin, M. T. D., Schultz, T. W., and Madden, J. C. (2008) Quantitative and mechanistic read across for predicting the skin sensitization potential of alkenes acting via Michael addition. *Chem Res Toxicol*, *21*, 513-520.
(106) Davies, M. J., and Truscott, R. J. W. (2001) Photo-oxidation of proteins and its role in cataractogenesis. *Journal of Photochemistry and Photobiology B-Biology*, *63*, 114-125.
(107) Lhiaubet-Vallet, V., Marin, M., Jimenez, O., Gorchs, O., Trullas, C., and Miranda, M. A. (2010) Filter-filter interactions. Photostabilization, triplet quenching and reactivity with singlet oxygen. *Photochemical & Photobiological Sciences*, *9*, 552-558.
(108) Wolken, W. A. M., ten Have, R., and van der Werf, M. J. (2000) Amino acid-catalyzed conversion of citral: cis-trans isomerization and its conversion into 6-methyl-5-hepten-2-one and acetaldehyde. *J Agric Food Chem*, *48*, 5401-5405.
(109) Wolken, W. A. M., Tramper, J., and van der Werf, M. J. (2004) Amino acid-catalysed retroaldol condensation: the production of natural benzaldehyde and other flavour compounds. *Flavour Fragr J*, *19*, 115-120.
(110) Costanzo, L. L., Deguidi, G., Condorelli, G., Cambria, A., and Fama, M. (1989) Molecular Mechanism of Drug Photosensitization .2. Photohemolysis Sensitized by Ketoprofen. *Photochem Photobiol*, *50*, 359-365.
(111) Bosca, F., Miranda, M. A., Carganico, G., and Mauleon, D. (1994) Photochemical and Photobiological Properties of Ketoprofen Associated with the Benzophenone Chromophore. *Photochem Photobiol*, *60*, 96-101.
(112) Kamide, R., and Matsushita, T. (2001) Five cases of photocontact dermatitis due to topical ketoprofen: photopatch testing and cross-reaction study. *Photodermatology Photoimmunology & Photomedicine*, *17*, 26-31.
(113) Hindsen, M., Zimerson, E., and Bruze, M. (2006) Photoallergic contact dermatitis from ketoprofen in southern Sweden. *Contact Dermatitis*, *54*, 150-157.
(114) Eriksson, L. A., Musa, K. A. K., and Matxain, J. M. (2007) Mechanism of photoinduced decomposition of ketoprofen. *Journal of Medicinal Chemistry*, *50*, 1735-1743.
(115) Nakagawa, M., Okajima, H., and Hino, T. (1977) PHOTOSENSITIZED OXYGENATION OF NB-METHOXYCARBONYLTRYPTAMINES - NEW PATHWAY TO KYNURENINE DERIVATIVES. *J Am Chem Soc*, *99*, 4424-4429.
(116) Sivaguru, J., Solomon, M. R., Poon, T., Jockusch, S., Bosio, S. G., Adam, W., and Turro, N. J. (2008) The reaction of singlet oxygen with enecarbamates: A mechanistic playground for investigating chemoselectivity, stereoselectivity, and vibratioselectivity of photooxidations. *Accounts Chem Res*, *41*, 387-400.
(117) Bakavoli, M., Sabzevari, O., and Rahimizadeh, M. (2007) H-Y zeolites induced heterocyclization: Highly efficient synthesis of substituted-quinazolin-4(3H)ones under microwave irradiation. *Chinese Chem Lett*, *18*, 533-535.

(118) Allen, J. M., Gossett, C. J., and Allen, S. K. (1996) Photochemical formation of singlet molecular oxygen in illuminated aqueous solutions of several commercially available sunscreen active ingredients. *Chemical Research in Toxicology*, *9*, 605-609.
(119) Balskus, E. P., Case, R. J., and Walsh, C. T. (2011) The biosynthesis of cyanobacterial sunscreen scytonemin in intertidal microbial mat communities. *Fems Microbiol Ecol*, *77*, 322-332.
(120) Walsh, C. T., and Balskus, E. P. (2008) Investigating the Initial Steps in the Biosynthesis of Cyanobacterial Sunscreen Scytonemin. *J Am Chem Soc*, *130*, 15260-+.
(121) Walsh, C. T., and Balskus, E. P. (2009) An Enzymatic Cyclopentyl[b]indole Formation Involved in Scytonemin Biosynthesis. *J Am Chem Soc*, *131*, 14648-+.
(122) Rivero, M. R., Adrio, J., and Carretero, J. C. (2002) Pauson-Khand reactions of electron-deficient alkenes. *Eur J Org Chem*, 2881-2889.
(123) Ezquerra, J., Pedregal, C., Lamas, C., Barluenga, J., Perez, M., GarciaMartin, M. A., and Gonzalez, J. M. (1996) Efficient reagents for the synthesis of 5-, 7-, and 5,7-substituted indoles starting from aromatic amines: Scope and limitations. *Journal of Organic Chemistry*, *61*, 5804-5812.
(124) Tripathi, V. K., Venkataramani, P. S., and Mehta, G. (1979) Addition of Nitrogen-Containing, Oxygen-Containing, and Sulfur-Containing Nucleophiles to Aryl Ethynyl Ketones. *J Chem Soc Perk T 1*, 36-41.
(125) Luche, J. L., Rodriguezhahn, L., and Crabbe, P. (1978) Reduction of Natural Enones in Presence of Cerium Trichloride. *J Chem Soc Chem Comm*, 601-602.
(126) Stevenson, P. J., McAlonan, H., Murphy, J. P., Nieuwenhuyzen, M., Reynolds, K., Sarma, P. K. S., and Thompson, N. (2002) 4-Phenyloxazolidin-2-ones and isoindolin-1-ones: chiral auxiliaries for Diels-Alder reactions of N-substituted 1,3-dienes. *J Chem Soc Perk T 1*, 69-79.
(127) Di Bussolo, V., Fiasella, A., Romano, M. R., Favero, L., Pineschi, M., and Crotti, P. (2007) Stereoselective synthesis of 2 3-unsaturated-aza-O-glycosides via new diastereoisomeric N-Cbz-imino glycal-derived allyl epoxidest. *Org Lett*, *9*, 4479-4482.
(128) Comins, D. L., and Killpack, M. O. (1992) Stereoselective Addition of (Triphenylsilyl)Magnesium Bromide to Chiral 1-Acyl-4-Methoxypyridinium Salts - Synthesis and Reactions of Enantiopure 1-Acyl-2-(Triphenylsilyl)-2,3-Dihydro-4-Pyridones. *J Am Chem Soc*, *114*, 10972-10974.
(129) Kozikowski, A. P., and Park, P. (1990) Synthesis of Streptazolin - Use of the Aza-Ferrier Reaction in Conjunction with the Inoc Process to Deliver a Unique but Sensitive Natural Product. *Journal of Organic Chemistry*, *55*, 4668-4682.
(130) Comins, D. L., and Foley, M. A. (1988) The Addition of Alkylzinc Iodides to 1-(Phenoxycarbonyl)-2,3-Dihydropyridinium Salts - a Synthesis of 2-Alkyl-Delta-3-Piperidines. *Tetrahedron Lett*, *29*, 6711-6714.
(131) Cazes, B., Ahmar, M., and Antras, F. (1999) Pauson-Khand reaction of activated olefins. *Tetrahedron Lett*, *40*, 5503-5506.
(132) Costa, M., and Mor, A. (1995) New Substituted Cyclopentenones by Coupling of Pauson-Khand and Michael-Type Reactions. *Tetrahedron Lett*, *36*, 2867-2870.
(133) Khand, I. U., Knox, G. R., Pauson, P. L., and Watts, W. E. (1971) Cobalt Induced Cleavage Reaction and a New Series of Arenecobalt Carbonyl Complexes. *Journal of the Chemical Society D-Chemical Communications*, 36-&.
(134) Khand, I. U., Knox, G. R., Pauson, P. L., Watts, W. E., and Foreman, M. I. (1973) Organocobalt Complexes .2. Reaction of Acetylenehexacarbonyldicobalt Complexes, (R1c2r2)Co2(Co)6, with Norbornene and Its Derivatives. *J Chem Soc Perk T 1*, 977-981.

(135) Magnus, P., and Principe, L. M. (1985) Origins of 1,2-Stereoselectivity and 1,3-Stereoselectivity in Dicobaltoctacarbonyl Alkene Alkyne Cyclizations for the Synthesis of Substituted Bicyclo[3.3.0]Octenones. *Tetrahedron Lett*, *26*, 4851-4854.
(136) Magnus, P., Exon, C., and Albaughrobertson, P. (1985) Dicobaltoctacarbonyl Alkyne Complexes as Intermediates in the Synthesis of Bicyclo[3.3.0]Octenones for the Synthesis of Coriolin and Hirsutic Acid. *Tetrahedron*, *41*, 5861-5869.
(137) Magnus, P., Principe, L. M., and Slater, M. J. (1987) Stereospecific Dicobalt Octacarbonyl Mediated Enyne Cyclization for the Synthesis of the Cytotoxic Sesquiterpene (+/-)-Quadrone. *Journal of Organic Chemistry*, *52*, 1483-1486.
(138) Labelle, B. E., Knudsen, M. J., Olmstead, M. M., Hope, H., Yanuck, M. D., and Schore, N. E. (1985) Synthesis of 11-Oxatricyclo[5.3.1.02,6]Undecane Derivatives Via Organometallic Cyclizations. *Journal of Organic Chemistry*, *50*, 5215-5222.
(139) Perez-Castells, J., Dominguez, G., Casarrubios, L., and Rodriguez-Noriega, J. (2002) Indole and quinoline synthesis via intramolecular Pauson-Khand reactions of enamines and allylamines. *Helv Chim Acta*, *85*, 2856-2861.
(140) Garcia, A., Rodriguez, D., Castedo, L., Saa, C., and Dominguez, D. (2001) Synthesis of fused rings at a pivotal nitrogen: tandem Heck reactions of N-vinyl-2-iodobenzamides. *Tetrahedron Lett*, *42*, 1903-1905.
(141) Sugihara, T., Yamada, M., Yamaguchi, M., and Nishizawa, M. (1999) The intra- and intermolecular Pauson-Khand reaction promoted by alkyl methyl sulfides. *Synlett*, 771-773.
(142) Brown, J. A., Irvine, S., Kerr, W. J., and Pearson, C. M. (2005) New odourless protocols for efficient Pauson-Khand annulations. *Org Biomol Chem*, *3*, 2396-2398.
(143) Krafft, M. E., and Bonaga, L. V. R. (2004) When the Pauson-Khand and Pauson-Khand type reactions go awry: a plethora of unexpected results. *Tetrahedron*, *60*, 9795-9833.
(144) Ahmar, M., Antras, F., and Cazes, B. (1999) Pauson-Khand reaction of activated olefins. *Tetrahedron Lett*, *40*, 5503-5506.
(145) Cuny, G. D., and Duval, E. (2004) Synthesis of substituted carbazoles and beta-carbolines by cyclization of diketoindole derivatives. *Tetrahedron Lett*, *45*, 5411-5413.
(146) Baran, P. S., and Shenvi, R. A. (2006) Total synthesis of (+/-)-chartelline C. *J Am Chem Soc*, *128*, 14028-14029.
(147) Larock, R. C., and Tian, Q. P. (1998) Palladium-catalyzed annulation of internal alkynes by arene-containing vinylic iodides and triflates. *Journal of Organic Chemistry*, *63*, 2002-2009.
(148) Incze, M., Moldvai, I., Temesvari-Major, E., Dornyei, G., Kajtar-Peredy, M., and Szantay, C. (2003) Chemistry of indoles carrying a basic function. Part 8: A new approach to the ergoline skeleton. *Tetrahedron*, *59*, 4281-4286.
(149) Tsunoda, T., Suzuki, M., and Noyori, R. (1980) Trialkylsilyl Triflates .6. Facile Procedure for Acetalization under Aprotic Conditions. *Tetrahedron Lett*, *21*, 1357-1358.
(150) Arthuis, M., Pontikis, R., and Florent, J. C. (2007) Tandem Heck-Suzuki-Miyaura reaction: Application to the synthesis of constrained analogues of combretastatin A-4. *Tetrahedron Lett*, *48*, 6397-6400.
(151) Player, M. R., Cheung, W. S., and Patch, R. J. (2005) A tandem Heck-carbocyclization/Suzuki-coupling approach to the stereoselective syntheses of asymmetric 3,3-(diarylmethylene)indolinones. *Journal of Organic Chemistry*, *70*, 3741-3744.

(152) Molander, G. A., and Felix, L. A. (2005) Stereoselective Suzuki-Miyaura cross-coupling reactions of potassium alkenyltrifluoroborates with alkenyl bromides. *Journal of Organic Chemistry*, *70*, 3950-3956.
(153) Larock, R. C., and Zhang, H. M. (2003) Synthesis of annulated gamma-carbolines and heteropolycycles by the palladium-catalyzed intramolecular annulation of alkynes. *Journal of Organic Chemistry*, *68*, 5132-5138.
(154) Baran, P. S., DeMartino, M. P., and Chen, K. (2008) Intermolecular enolate heterocoupling: Scope, mechanism, and application. *J Am Chem Soc*, *130*, 11546-11560.
(155) Kim, J. W., Lee, J. J., Lee, S. H., and Ahn, K. H. (1998) Stereoselective homocoupling of enolate anion promoted by hypervalent iodine compound. *Synthetic Commun*, *28*, 1287-1292.
(156) Rivero, M. R., Alonso, I., and Carretero, J. C. (2004) Vinyl sulfoxides as stereochemical controllers in intermolecular Pauson-Khand reactions: Applications to the enantioselective synthesis of natural cyclopentanoids. *Chem-Eur J*, *10*, 5443-5459.
(157) Shambayati, S., Crowe, W. E., and Schreiber, S. L. (1990) N-Oxide Promoted Pauson-Khand Cyclizations at Room-Temperature. *Tetrahedron Lett*, *31*, 5289-5292.
(158) Krafft, M. E., Wilson, A. M., Dasse, O. A., Bonaga, L. V. R., Cheung, Y. Y., Fu, Z., Shao, B., and Scott, I. L. (1998) Dienes from the thermolysis of dicobalthexacarbonyl-complexed enynes: Mechanistic insight. *Tetrahedron Lett*, *39*, 5911-5914.
(159) Mukai, C., Sonobe, H., Kim, J. S., and Hanaoka, M. (2000) Pauson-Khand reaction of optically active 6,7-bis(tert-butyldimethylsiloxy)non-1-en-8-ynes. *Journal of Organic Chemistry*, *65*, 6654-6659.
(160) Estrada, E., Patlewicz, G., Chamberlain, M., Basketter, D., and Larbey, S. (2003) Computer-aided knowledge generation for understanding skin sensitization mechanisms: The TOPS-MODE approach. *Chemical Research in Toxicology*, *16*, 1226-1235.
(161) Council Directive of the EC, E. U. (2008) Annex VI: List of preservatives which cosmetic products may contain, In *76/768/EEC* (Union, E., Ed.) pp 112-120.
(162) Goossens, A., Garcia-Gavin, J., Vansina, S., Kerre, S., and Naert, A. (2010) Methylisothiazolinone, an emerging allergen in cosmetics? *Contact Dermatitis*, *63*, 96-101.
(163) Lundov, M. D., Thyssen, J. P., Zachariae, C., and Johansen, J. D. (2010) Prevalence and cause of methylisothiazolinone contact allergy. *Contact Dermatitis*, *63*, 164-167.
(164) Lundov, M. D., Zachariae, C., and Johansen, J. D. (2011) Methylisothiazolinone contact allergy and dose-response relationships. *Contact Dermatitis*, *64*, 330-336.
(165) Amaro, C. A., C., Santos, R., and Cardoso, J. (2011) Contact allergy to methylisothiazolinone in a deodorant. *Contact Dermatitis*, *64*, 298-299.
(166) Garciapichel, F., Sherry, N. D., and Castenholz, R. W. (1992) Evidence for an Ultraviolet Sunscreen Role of the Extracellular Pigment Scytonemin in the Terrestrial Cyanobacterium Chlorogloeopsis Sp. *Photochemistry and Photobiology*, *56*, 17-23.
(167) Hader, D. P., and Sinha, R. P. (2008) UV protectants in cyanobacteria. *Plant Sci*, *174*, 278-289.
(168) Jonckers, T. H. M., van Miert, S., Cimanga, K., Bailly, C., Colson, P., De Pauw-Gillet, M. C., van den Heuvel, H., Claeys, M., Lemiere, F., Esmans, E. L., Rozenski, J., Quirijnen, L., Maes, L., Dommisse, R., Lemiere, G. L. F., Vlietinck, A., and Pieters, L. (2002) Synthesis, cytotoxicity, and antiplasmodial and antitrypanosomal activity of new neocryptolepine derivatives. *Journal of Medicinal Chemistry*, *45*, 3497-3508.

Results from the photolysis of 4,4'-di-tert-butyldibenzoylmethane 4.1c

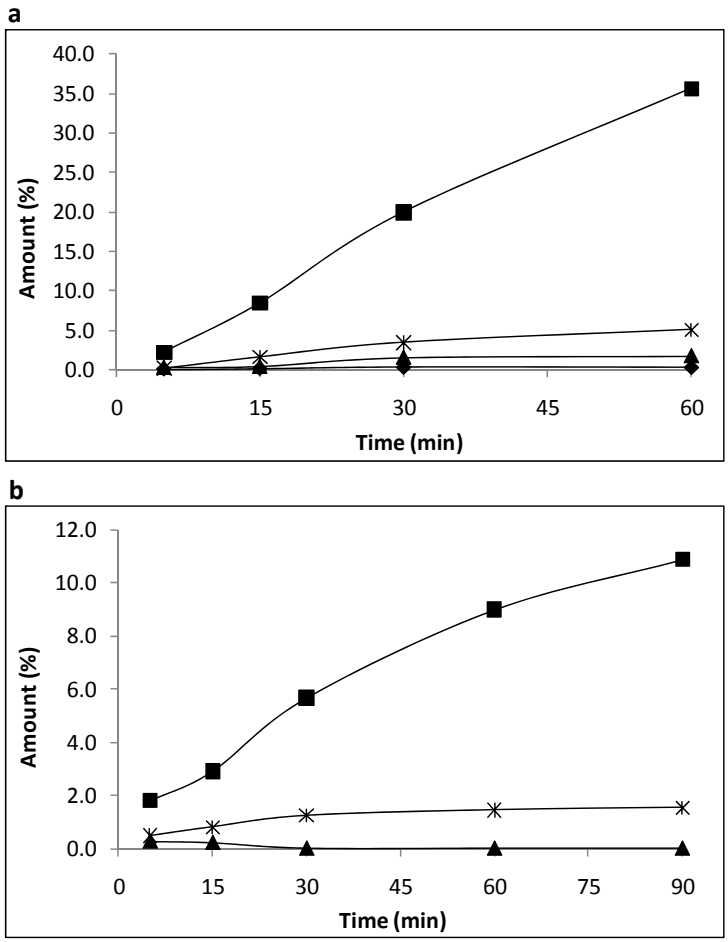

Figure AI.1. Formation of photodegradation products from the photolysis experiments with dibenzoylmethane **4.1c**. The amount is in relation to the initial concentration of dibenzoylmethane **4.1c**. The following photoproducts were identified: **4.2a** (▲), **4.3c** (♦) and **4.4a** (■), **4.5a**+**4.6a** (ж). (a) Photolysis in cyclohexane. (b) Photolysis in EtOH.

Results from the photolysis of 4,4'-dimethoxybutyldibenzoylmethane 4.1d

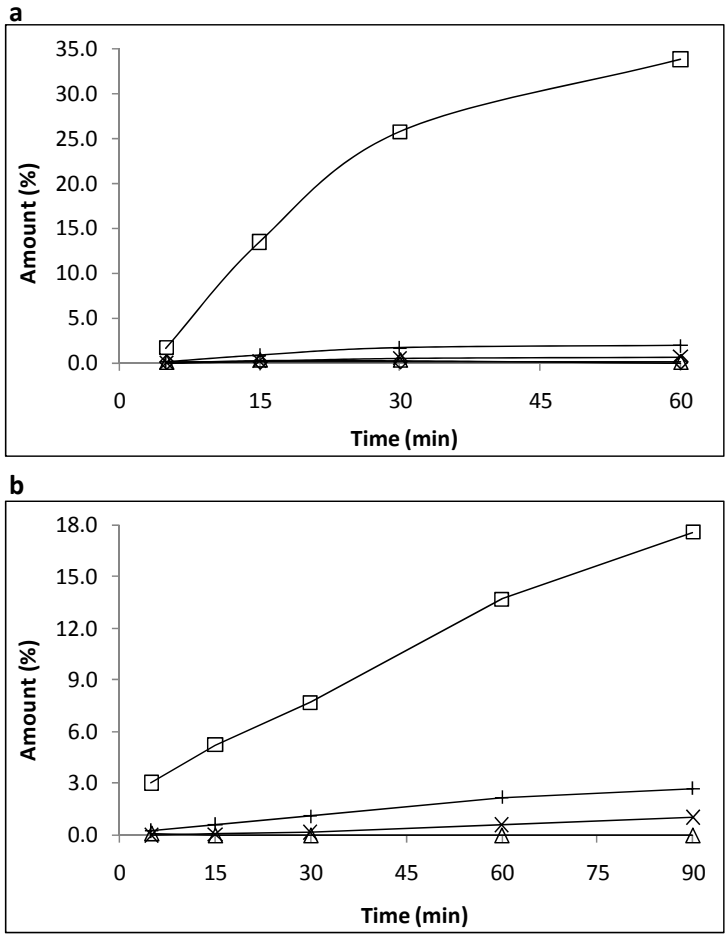

Figure AI.2. Formation of photodegradation products from the photolysis experiments with dibenzoylmethane **4.1d**. The amount is in relation to the initial concentration of dibenzoylmethane **1d**. The following degradation products were identified: **4.2b** (**Δ**), **4.3d** (◊), **4.4b** (□), **4.5b** (**×**) and **4.6b** (**+**). (a) Photolysis in cyclohexane. (b) Photolysis in EtOH.

[^{3}H]-Thymidine incorporation and SI values for compounds assessed in the LLNA

Table AI.1. LLNA responses for compounds 4.1a, b and 4.2a-d

test material and concentration (% w/v)	[^{3}H]-thymidine incorporation (DPM/lymph node)	SI
4.1a		
control	788	
1% (0.032 M)	1083	1.4
10% (0.32 M)	1448	1.8
30% (0.97 M)	1243	1.6
60% (1.9 M)	1059	1.3
80% (2.6 M)	902	1.2
4.1b		
control	887	
1% (0.038 M)	787	0.9
10% (0.38 M)	1481	1.7
30% (1.1 M)	2396	2.7
60% (2.3 M)	5649	6.4
80% (3.0 M)	5587	6.3
4.2a		
control	481	
0.01% (2.5×10^{-4} M)	474	1.0
0.1% (0.0025 M)	573	1.2
0.5% (0.013 M)	3518	7.3
2.5% (0.063 M)	6137	12.8
10% (0.25 M)	14758	30.7
4.2b		
control	920	
0.01% (2.9×10^{-4} M)	1010	1.1
0.1% (0.0029 M)	1844	2.0
0.5% (0.014 M)	8162	8.9
2.5% (0.072 M)	12477	13.6
10% (0.29 M)	16196	17.6
4.2c		
control	656	
0.05% (0.0026 M)	1166	1.8
0.25% (0.013 M)	1723	2.6
1% (0.051 M)	4452	6.8
5% (0.26 M)	11248	17.2
30% (1.5 M)	19616	29.9
4.2d		
control	660	
0.01% (5.1×10^{-4} M)	824	1.3
0.1% (0.0051 M)	998	1.5
0.5% (0.025 M)	4040	6.1
2.5% (0.013 M)	12505	19.0
10% (0.51 M)	13133	19.9

Table AI.2. LLNA responses for benzils 4.3a and 4.3c-e

test material and concentration (% w/v)	[^{3}H]thymidine incorporation (DPM/lymph node)	SI
4.3a		
control	548	
0.1% (0.0034 M)	549	1.0
0.5% (0.017 M)	221	0.4
1% (0.034 M)	547	1.0
5% (0.17 M)	540	1.0
10 (0.34 M))	565	1.0
4.3c		
control	304	
0.1% (0.0031 M)	529	1.7
0.5% (0.016 M)	358	1.2
1% (0.031 M)	1067	3.5
5% (0.16 M)	519	1.7
10% (0.31 M)	461	1.5
4.3d		
control	396	
0.1% (0.0037 M)	282	0.7
0.25% (0.0092 M)	401	1.0
0.5% (0.018 M)	377	1.0
1% (0.037 M)	400	1.0
2.5% (0.092 M)	387	1.0
4.3e		
control	396	
0.1% (0.0037 M)	282	0.7
0.25% (0.0092 M)	401	1.0
0.5% (0.018 M)	377	1.0
1% (0.037 M)	400	1.0
2.5% (0.092 M)	387	1.0

APPENDIX II: SUPPLEMENTARY INFORMATION TO CHAPTER 7

Experimental procedures for synthesis of compounds discussed in section 7.1

Instrumentation. Chromatographic separations were performed using Merck silica gel Geduran Si 60 (0.063-0.200 mm) and Sigma-Aldrich hexane mixture of isomers (bp 68-70° C). ^{1}H and ^{13}C NMR spectra were recorded in on a JEOL eclipse+ 400 MHz spectrometer at 400 and 100 MHz, respectively. Chemical shifts (δ) are reported in ppm with the solvent residual peaks as internal standard ($CHCl_3$ δ 7.26, $CDCl_3$ δ 77.0).

*2-(Trimethylsilylethynyl)aniline (**7.A4**).* A procedure was adapted from the literature (*123*) as follows: 2-Iodoaniline (4.4 g, 20 mmol) , $Pd(PPh_3)_2Cl_2$ (0.72 g, 1.0 mmol), and CuI (0.19 g, 1.0 mmol) were added to an oven-dried flask under argon. Dry Et_3N (8.3 mL, 60 mmol) was added to the flask and the stirred suspension was cooled to 0 °C, and trimethylsilylacetylene (3.1 mL, 22 mmol) was added dropwise. The mixture was stirred at RT under argon for 16 h and the solvent was then removed at reduced pressure. Diethylether was added to the resulting residue and the mixture was filtered through celite, washed with saturated aqueous NaCl and dried over Na_2SO_4. Thereafter, the solvent was removed at reduced pressure and the crude product was purified by column chromatography on silica gel (ethyl acetate/hexane 1:9), which afforded the product (3.7 g, 97%). The obtained NMR was according to reference (*168*).

*1-Phenyl-3((2-(trimethylsilylethynyl)phenyl)amino)prop-2-en-1-one (**7.A5**).* The *N*-aryl-enaminone (**7.A5**) was synthesized using the literature procedure described by Vijay et al (*124*). Aniline **7.A4** (0.57 g, 3.0 mmol) and 1-phenyl-2-propyn-1-one (0.39 g, 3.0 mmol) in dry toluene (10 mL) was stirred at 90 °C. After 21 h the mixture was allowed to reach room temperature and the solvent was removed at reduced pressure. The crude product was purified by column chromatography on silica gel (toluene/hexane 1:1), which afforded the product as a yellow solid (0.61 g, 64%). ^{1}H NMR δ: 12.50 (d, *NH*, *J* = 11.7 Hz), 7.96 (d, 2H, *J* = 7.0 Hz), 7.60-7.40 (m, 5H), 7.30 (dd, 1H, *J* = 8.0, 7.7 Hz), 7.17 (d, 1H, *J* = 8.4 Hz), 6.96 (dd, 1H, *J* = 7.7, 7.4 Hz), 6.10 (d, 1H, *J* = 8.0), 0.41 (s, 9H); ^{13}C NMR δ: 190.2, 142.6, 142.4, 139.3, 132.9, 131.6, 129.8, 128.5, 127.5, 122.4, 112.4, 112.2, 102.8, 99.8, 94.80, 0.1.

*N-(3-Oxo-3-phenylprop-1-en-1-yl)-N-(2-(trimethylsilylethynyl)phenyl)acetamide (**7.A6**).* A solution of *N*-aryl-enaminone **7.A5** (0.32 g, 0.40 mmol), Et_3N (75 µL, 0.50 mmol), acetyl chloride (35 µL, 0.50 mmol), and a catalytic amount of 4-dimethylaminopyridine in dichloromethane (5 mL) was refluxed for 5h. The mixture was allowed to reach room temperature and 20 mL of 5% HCl was added and the organic layer was separated. The water phase was extracted twice with dichloromethane. The organic extracts were combined and washed with saturated aqueous NaCl, dried over K_2CO_3, filtered and concentrated at reduced pressure. The crude products was purified by column chromatography on silica gel (ethyl acetate/hexane 1:9), which afforded the product (0.13 g, 90%). ^{1}H NMR δ: 8.73 (d, 1H, *J* = 13.9 Hz), 7.73-7.22 (m, 9H), 5.65 (d, 1H, *J* = 13.9 Hz), 1.98 (s, 3H), 0.15 (s, 9H); ^{13}C NMR δ:191.1, 169.6, 142.4, 140.5, 138.8, 133.8, 132.3, 130.4, 129.6, 128.8, 128.5, 128.2, 128.1, 123.1, 107.2, 99.6, 23.0, -0.3.

*Experimental procedure for preparation of enamides **7.A11** and **7.A12**.* A literature procedure was used as follows (*140*): A solution of hydrocinnamaldehyde (1.3 mL, 10 mmol) in 1 mL dry THF was added to a cooled (-10 °C) solution of aniline **7.A4** or **7.A10** (8.5 mmol) in 5 mL of dry THF over 4 Å molecular sieves. After stirring under N_2 for 4.5 h while the mixture reached room temperature, Et_3N (1.4 mL, 10 mmol) and acetyl chloride (0.70 mL, 9.0 mmol) in dry THF (3 mL) was added. The mixture was stirred for another hour at room temperature. The sieves were then filtered off and rinsed with dichloromethane. The resulting organic solution was washed with water, dried over Na_2SO_4, and concentrated at reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetat/hexane 1:4), which afforded **7.A11** in 5% and **7.A12** in 24%.

*N-(3-Phenylprop-1-en-1-yl)-N-(2-(trimethylsilylethynyl)phenyl)acetamide (**7.A11**)*. ^{1}H NMR δ: 7.60-7.10 (m, 10H), 4.47 (m, 1H), 3.34 (m, 2H), 1.86 (s, 3H), 0.22 (s, 9H); ^{13}C NMR δ: 168.4, 142.0, 140.9, 133.5, 130.0, 129.4, 128.8, 128.7, 128.44, 128.38, 126.1, 123.5, 112.0, 100.6, 100.4, 36.3, 22.7, -0.2.

*N-(2-Ethynylphenyl)-N-(3-phenylprop-1-en-1-yl)acetamide (**7.A12**).* ^{1}H NMR δ: 7.65-7.10 (m, 10H), 4.47 (m, 1H), 3.34 (m, 2H), 3.22 (s, 1H), 1.87 (s, 3H), 0.22 (s, 9H); ^{13}C NMR δ: 168.5, 141.9, 140.8, 134.3, 130.5, 129.6, 128.8, 128.7, 128.5, 128.4, 126.1, 122.6, 112.3, 82.5, 79.1, 36.3, 22.9.

Paper I

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Paper III

Investigation of the Sunscreen Octocrylene's Interaction with Amino Acid Analogs in the Presence of UV radiation

Isabella Karlsson, Jerker Mårtensson, and Anna Börje.

Submitted for publication

Paper IV

Ketoprofen Induced Formation of Amino Acid Photoadducts: Possible Explanation for Photocontact Allergy to Ketoprofen

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