

Clinical and experimental studies of octocrylene's allergenic potency

Isabella Karlsson¹, Katrien Vanden Broecke², Jerker Mårtensson³, An Goossens² and Anna Börje¹

¹Dermatochemistry and Skin Allergy, Department of Chemistry, University of Gothenburg, SE-412 96, Gothenburg, Sweden, ²Department of Dermatology, University Hospital Saint-Raphaël, Katholieke Universiteit, Leuven, B-3000, Belgium, and ³Department of Chemical and Biological Engineering/Organic Chemistry, Chalmers University of Technology, SE-412 96, Gothenburg, Sweden

doi:10.1111/j.1600-0536.2011.01899.x

Summary

Background. Reports of positive patch test and photopatch test reactions to the chemical ultraviolet filter octocrylene have increased during the last decade. Little is known about the reason for octocrylene's allergenic activity.

Objectives. To present and discuss the results of patch tests and photopatch tests with octocrylene, and to investigate the possible cause of its allergenic properties.

Methods. Results of patch tests and photopatch tests with octocrylene in patients with adverse skin reactions to sunscreen products and/or ketoprofen were collected. The allergenic potency of octocrylene was investigated in the murine local lymph node assay (LLNA). Chemical reactivity assays were used to mimic octocrylene's interaction with biomolecules.

Results. We report 23 cases of positive test reactions to octocrylene (5 patch test and 18 photopatch). Notably, many of these patients also had positive photopatch test reactions to ketoprofen and benzophenone-3. Octocrylene was shown to be a moderate sensitizer in the LLNA, and it reacted with amines such as lysine, but not with thiols such as cysteine.

Conclusions. The clinical studies show that octocrylene is both a photocontact allergen and a contact allergen. Octocrylene's ability to cause contact allergy is probably attributable to its reactivity towards lysine. To be able to understand why octocrylene causes photocontact allergy, further studies are needed.

Key words: amino acid binding; benzophenone; contact allergy; ketoprofen; local lymph node assay; octocrylene; photocontact allergy.

The rising public awareness of the harmful effects of the sun's ultraviolet (UV) radiation has led to an increase in the use of sunscreens (1, 2). Octocrylene (INCI) (CAS 6197-30-4) (Fig. 1), a hydrophobic compound (Log P 6.9) (3) within the cinnamate family, is a chemical UV filter that is considered to be highly stable,

providing protection against UVB as well as short UVA wavelengths (4). It has also been suggested to stabilize other UV filters, such as butyl methoxydibenzoylmethane (INCI) (CAS 70356-09-1), against photodegradation (5). These attractive properties have contributed to its widespread use in sunscreens and cosmetic preparations during the last 10 years, and it is approved by EU (6) and Food and Drug Administration (FDA) (7) in concentrations up to 10%.

The first cases of contact allergy and photocontact allergy caused by octocrylene were reported in 2003 by Carrotte-Lefebvre et al. (8). The number of reports on allergic reactions to octocrylene has increased since then (9–12); for example, an Italian multicentre study from 2004 to 2006 in 1082 patients reported 23 cases of positive patch test and/or photopatch test reactions

Correspondence: Professor Anna Börje, Dermatochemistry and Skin Allergy, Department of Chemistry, University of Gothenburg, Kemiv. 10, 412 96 Gothenburg, Sweden. Tel: +46 31 786 9012; Fax: +46 31 772 3840. E-mail: aborje@chem.gu.se

Conflicts of interest: The authors have declared no conflicts.

Funding: The Centre for Skin Research at the University of Gothenburg and The Swedish Research Council.

Accepted for publication 29 January 2011

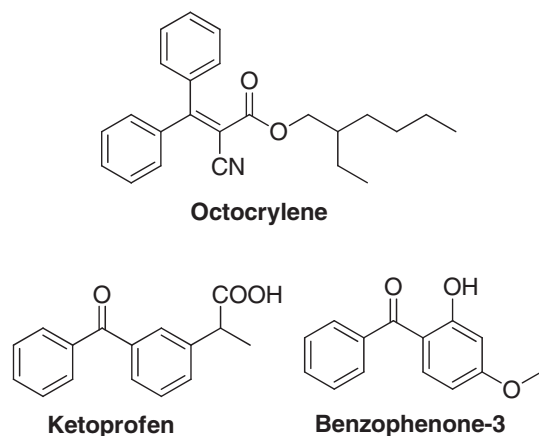


Fig. 1. Chemical structures of the compounds discussed in this study.

to octocrylene, which made it the prime allergen of the chemical UV filters in that study (13). In 2010, clinics from France and Belgium, gathered under REVIDAL (14), published an article in which they reported as many as 50 cases of positive patch test and/or photopatch test reactions to octocrylene over a period of 2 years (15). The use of octocrylene in sunscreen products and cosmetics is increasing, and sensitization to octocrylene is therefore expected to increase in the near future (10, 15). In the present article, we report and discuss another 23 cases of positive patch test and photopatch test reactions to octocrylene, diagnosed at the Department of Dermatology in Leuven, Belgium during the years 2000–2010. Furthermore, we have investigated the properties of octocrylene that render it allergenic, that is, able to cause contact allergy. The sensitizing capacity of octocrylene was studied with the local lymph node assay (LLNA) in mice. To mimic protein interactions, the chemical reactivity of octocrylene towards the nucleophiles benzylamine, protected lysine and protected cysteine was investigated.

Materials and Methods

Clinical studies

From January 2000 to September 2010, 172 Caucasian patients with (suspected) adverse skin reactions to sunscreen products and/or ketoprofen were patch tested at the Department of Dermatology, University Hospital Saint-Raphaël, Katholieke Universiteit, Leuven, Belgium. Ninety of these patients were also photopatch tested with sunscreen agents, including octocrylene (10% in petrolatum). From May 2008, 43 of the patients were

patch and photopatch tested with the European baseline photopatch tests series, also including non-steroidal anti-inflammatory agents, such as ketoprofen. The tests were performed 1 month or more after complete resolution of the dermatitis. The patch tests were performed on one side of the patient's back on D0, with patch test chambers (Van der Bend, Brielle, The Netherlands), mounted on Micropore™ (3M Health Care, Borken, Germany) and covered with Mefix® (Mölnlycke Health Care, Göteborg, Sweden). The patch tests were removed and read at D2 and again at D4 and D7, according to International Contact Dermatitis Research Group recommendations (16). On the opposite side of the back, the chambers with the different substances were similarly applied at D0, and irradiated (5 J/cm²) at D2 with UVA, using Philips Performance 100W-R lamps. The photopatch tests were read immediately after irradiation (D2) and then again at 2 and 5 days after irradiation (D4 and D7).

Chemicals

Unless otherwise indicated, reagents were used without further purification. Dibutylphthalate, ethyl acetate, octocrylene (2-ethylhexyl 2-cyano-3,3-diphenyl-2-propenoate; IUPAC), hexane mixture of isomers, *N*_α-acetyl-L-lysine methyl ester hydrochloride (*N*_α-Ac-L-Lys-Me-ester•HCl) and phosphate-buffered saline (PBS) tablets were from Sigma-Aldrich Chemie (Steinheim, Germany). *N*-Acetyl-L-cysteine-methyl ester (*N*-Ac-L-Cys-Me-ester) was from Fluka, Sigma-Aldrich Chemie (Steinheim, Germany). Acetone was from Merck (Darmstadt, Germany), benzophenone-3 was from Merck-Schuchardt (Hohenbrunn, Germany), olive oil was from Apoteket AB (Göteborg, Sweden), and [³H]thymidine was from Amersham Biosciences (Little Chalfont, UK). Ethanol (EtOH), 95.1–96.9 vol.%, was from Solveco (Rosersberg, Sweden), and acetonitrile (ACN), liquid chromatography (LC)/mass spectrometry (MS) Grade, was from Fischer Scientific (Loughborough, UK). Benzylamine was from Sigma-Aldrich Chemie, and was purified by distillation under reduced pressure. The carbonate buffer (pH 10.0) contained 25 mM sodium carbonate and 25 mM sodium hydrogen carbonate. The purity of octocrylene was determined to be >99% [gas chromatography (GC)/MS] pure before investigation of its sensitizing capacity.

Instrumentation and mode of analysis

β-Scintillation counting was performed with a Beckman LS 6000TA instrument.

GC/MS analyses were performed by electron ionization (70 eV) on a Hewlett-Packard model 5973 mass

spectrometer (scanned m/z 50–500), connected to a gas chromatograph (Hewlett-Packard model 6890). The gas chromatograph was equipped with an on-column inlet and an HP-5MSI fused silica capillary column (30 m $\times$ 0.25 mm, 0.25 μ m film thickness). Helium was used as carrier gas, the flow rate was 1.2 ml/min, and the injection volume was 1 μ l. The column temperature was 100°C at injection, raised to 200°C at a rate of 5°C/min, then raised from 200°C to 270°C at a rate of 15°C/min, and finally held at 270°C for 20 min.

High-performance liquid chromatography (HPLC)/MS analyses were performed by electrospray ionization on a Hewlett-Packard 1100 HPLC/MS instrument. The system included a vacuum degasser, a binary pump, an autoinjector, a column thermostat, a diode array detector, and a single quadrupole mass spectrometer. The electrospray interface was used with the following spray chamber settings: nebulizer pressure, 35 psig (pounds-force per square inch gauge); capillary voltage, 3000 V; drying gas temperature, 350°C; and drying gas flow rate, 12 l/min. For mass spectral analysis, the mass spectrometer was used in the scan mode, detecting ions with m/z values ranging from 50 to 1000. Mass spectral analysis was performed in positive ionization mode with a fragmenter voltage of 70 V. A HyPURITY C18 column (100 $\times$ 2.1 mm, 3- μ m particles; Thermo Hypersil-Keystone, Bellafonte, PA, USA) was used, and the column temperature was set to 40°C. Mobile phase A consisted of 0.1% formic acid in Milli-Q water, and mobile phase B consisted of 0.1% formic acid in ACN.

Chromatographic separations were performed with Merck silica gel Geduran Si 60 (0.063–0.200 mm).

^1H -nuclear magnetic resonance (NMR) and ^{13}C -NMR spectra were recorded on a JEOL Eclipse + 400-MHz spectrometer, with CD_3CN as solvent. Chemical shifts (δ) are reported in ppm relative to CD_3CN at δ 1.94 and δ 118.2 for ^1H and ^{13}C , respectively.

Sensitization experiments in mice

The sensitizing potency of octocrylene was investigated with the LLNA (17). The animal procedure was approved by the local ethics committee. Octocrylene was tested in the following concentrations (wt/vol): 1.0% (0.028 M), 2.5% (0.069 M), 5.0% (0.14 M), 15% (0.42 M), and 30% (0.83 M). Female CBA/CA mice in groups of three received 25 μ l of a solution of the test compound, dissolved in the vehicle, acetone/olive oil (4:1 vol/vol), on the dorsum of the ears daily for three consecutive days. A control group with three mice was treated in the same way, but with vehicle alone. All mice were injected intravenously 5 days after the first treatment, with 250 μ l of PBS containing

20 μCi of [^3H]thymidine. Five hours later, the mice were killed. The draining lymph nodes were excised and pooled for each group, and a single-cell suspension of lymph node cells was prepared. The thymidine incorporation was measured by β -scintillation counting. Results are expressed as the mean dpm/lymph node for each experimental group and as stimulation index (SI). The SI is defined as the ratio between dpm/lymph node for the test group and that for the control group. If the compound at one or more concentrations causes an SI greater than 3, it is considered to be positive in the LLNA. The EC3 value, the estimated concentration required to induce an SI of 3, was calculated by linear interpolation (18). The sensitizing potency of the test compound was classified according to the following: <0.1%, extreme; $\geq$ 0.1% to <1%, strong; $\geq$ 1% to <10%, moderate; and $\geq$ 10% to 100%, weak (19).

Octocrylene chemical reactivity study

Stability of octocrylene (10 mM) in ACN/carbonate buffer pH 10.0 (3:1). Octocrylene (7.2 mg, 0.020 mmol) was dissolved in ACN/carbonate buffer pH 10.0 (3:1) (2.0 ml) at room temperature. At specific intervals (0.17, 3.0, 5.0 and 21 hr), aliquots of 10 μ l were removed and diluted with 10 μ l of EtOH and 180 μ l of ACN/carbonate buffer pH 10.0 (3:1) prior to HPLC/MS analysis.

Reactivity of octocrylene (10 mM) towards benzylamine (100 mM) in ACN/carbonate buffer pH 10.0 (3:1). Benzylamine (22 μ l, 22 mg, 0.20 mmol) was added to a stirred solution of octocrylene (7.2 mg, 0.020 mmol) and dibutylphthalate (internal standard, 5.3 μ l, 5.6 mg, 0.020 mmol) in ACN/carbonate buffer pH 10.0 (3:1) (2.0 ml) at room temperature. At specific intervals (0.10, 1.7, 3.2, 4.8, 6.3 and 7.9 hr), aliquots of 10 μ l were removed and diluted with 10 μ l of EtOH and 180 μ l of ACN/carbonate buffer pH 10.0 (3:1) prior to HPLC/MS analysis.

Reactivity of octocrylene (10 mM) towards benzylamine (100 mM) in EtOH. Benzylamine (22 μ l, 22 mg, 0.20 mmol) was added to a stirred solution of octocrylene (7.2 mg, 0.020 mmol) and dibutylphthalate (internal standard, 5.3 μ l, 5.6 mg, 0.020 mmol) in EtOH (2.0 ml) at room temperature. At specific intervals (0.10, 1.7, 3.2, 4.8, 6.3 and 8.1 hr), aliquots of 10 μ l were removed and diluted with 190 μ l of ACN/carbonate buffer pH 10.0 (3:1) prior to HPLC/MS analysis.

Reactivity of octocrylene (10 mM) towards N_α -Ac-L-Lys-Me-ester (100 mM) in ACN/carbonate buffer pH 10.0 (3:1). N_α -Ac-L-Lys-Me-ester $\cdot\text{HCl}$ (23 mg, 0.20 mmol) and NaOH (8.0 mg, 0.20 mmol) were dissolved in carbonate buffer

pH 10.0 (0.5 ml). This solution was then added to a stirred solution of octocrylene (7.2 mg, 0.020 mmol) and dibutylphthalate (internal standard, 5.3 µl, 5.6 mg, 0.020 mmol) in ACN (1.5 ml) at room temperature. At specific intervals (0.10, 1.7, 3.2, 4.7, 6.2 and 7.7 hr), aliquots of 10 µl were removed and diluted with 10 µl of EtOH and 180 µl of ACN/carbonate buffer pH 10.0 (3:1) prior to HPLC/MS analysis.

Reactivity of octocrylene (10 mM) towards *N*-Ac-L-Cys-Me-ester (100 mM) in EtOH. *N*-Ac-L-Cys-Me-ester (35 mg, 0.20 mmol) and octocrylene (7.2 mg, 0.020 mmol) were dissolved in EtOH (2.0 ml) at room temperature. At specific intervals (10 min, 2 hr, 6 hr, and 9 days), aliquots of 10 µl were removed and diluted with 190 µl of ACN/carbonate buffer pH 10.0 (3:1) prior to HPLC/MS analysis.

HPLC/MS analysis of the reaction mixtures. For each sample, aliquots of 5 µl were injected onto the column and eluted with a gradient flow of 0.40 ml/min. A linear gradient from 10% to 100% mobile phase B in 15 min was followed by 5 min of isocratic elution with 100% mobile phase B. The column was equilibrated with 10% mobile phase B for 10 min between each run. Octocrylene and the internal standard (dibutylphthalate) were detected with HPLC/UV at 305 and 254 nm, respectively. Quantification of octocrylene and dibutylphthalate was performed with standard curves made in close proximity in time to each reactivity experiment.

Purification of benzophenone-*N*-benzylimine. The reaction mixture from the reactivity experiment with octocrylene and benzylamine in EtOH was purified by column chromatography on silica gel (5% ethyl acetate in hexanes). The identity of the compound was verified by inspection of NMR and GC/MS spectra, which were in accordance with those reported by Cainelli et al. (20).

Synthesis of benzophenone-*N*_α-Ac-L-Lys-Me-ester-imine. Octocrylene (59 mg, 0.16 mmol) was dissolved in ACN (6 ml) and added to a stirred solution of *N*_α-Ac-L-Lys-Me-ester•HCl (190 mg, 0.80 mmol) and NaOH (32.0 mg, 0.80 mmol) in carbonate buffer pH 10.0 (2.0 ml). After 9 days of stirring at room temperature, the mixture was dried three times over MgSO₄, filtered each time, and finally concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (solvent gradient: hexanes/ethyl acetate 1:1, then hexanes/ethyl acetate 1:2, and then 100% ethyl acetate), which afforded the product as a colourless oil (10 mg, 18%). ¹H-NMR (CD₃CN) δ: 1.32–1.41 (m, 2H), 1.54–1.76 (m, 4H), 1.86 (s, 3H), 3.29 (t, 2H, *J* = 6.8), 3.62 (s, 3H), 4.29 (dt,

1H, *J* = 8.0, 5.3), 6.66 (d, NH, *J* = 5.3), 7.16 (ddd, 2H, *J* = 7.6, 2.6, 1.4), 7.31–7.43 (m, 3H), 7.43–7.53 (m, 3H), 7.60 (ddd, 2H, *J* = 7.6, 2.6, 1.4). ¹³C-NMR (CD₃CN) δ: 22.6, 24.2, 31.2, 32.0, 52.4, 53.2, 53.9, 128.5, 128.9, 128.9, 129.2, 129.4, 130.6, 137.9, 140.9, 167.4, 168.2, 173.8.

Results

Clinical studies

Of the 172 patients, 23 (15 females and 8 males) reacted positively to octocrylene (Table 1, in which only the

Table 1. Results from patch and photopatch tests with octocrylene, ketoprofen and benzophenone-3 for the 23 octocrylene-positive patients

Case	Age/ gender	History of topical NSAID use	Octocrylene		Ketoprofen		Benzophenone-3	
			PT	PPT	PT	PPT	PT	PPT
1*	41/F	Yes	–	++	–	+++	–	++
2*	71/M	Yes	–	+	–	++	–	–
3†	56/F	?	+	+	NT	NT	–	–
4†	44/M	?	–	+	NT	NT	–	–
5*	41/F	Yes	–	++	–	++	–	+
6†	48/F	No	–	++	NT	NT	–	–
7*	38/M	Yes	–	++	++	+++	–	+
8†	7/F	No	–	+	NT	NT	–	–
9‡	41/F	Yes	–	+	+	++	–	+
10†	3/F	No	+	+	–	–	–	–
11†	11/F	No	+	NT	NT	NT	–	–
12*	43/F	Yes	–	++	–	+++	–	+
13*	29/F	Yes	–	+	–	++	–	–
14*	33/M	Yes	+	+++	–	+++	–	++
15‡	54/M	Yes	–	+	–	++	–	+
16*	31/M	Yes	–	+++	–	+++	–	++
17*	36/F	Yes	+	+	–	+++	–	+++
18*	53/F	Yes	–	+++	–	+++	–	+
19*	42/F	Yes	–	++	–	+++	–	++
20‡	33/F	Yes	–	++	–	+++	–	++
21*	24/M	Yes	–	++	–	++	–	+
22*	24/M	Yes	–	+	–	+++	–	+
23*	32/F	Yes	–	++	–	++	–	++

F, female; M, male; NT, not tested; PT, patch test; PPT, photopatch test; NSAID, non-steroidal anti-inflammatory drugs.

Reactions are graded according to the International Contact Dermatitis Research Group recommendations (16): +++ = vesicles, papules, erythema, and infiltrate; ++ = papules, erythema, and infiltrate; + = erythema and infiltrate.

*Patients used or reacted to ketoprofen-containing Fastum® gel previously, and presented later with sunscreen intolerance.

†Intolerance to sunscreen product; no use of ketoprofen previously.

‡Reaction to ketoprofen previously, but no known sunscreen intolerance.

Table 2. [³H]Thymidine incorporation (dpm/lymph node) and stimulation index (SI) values for octocrylene tested in the local lymph node assay

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)

highest grading of the reactions is noted). Five of the 23 octocrylene-positive patients reacted in the patch test, whereas 18 patients reacted only in the photopatch test. The five contact-allergic cases were: an 11-year-old girl who was not photopatch tested with octocrylene, as she had already reacted in the patch test¹ (no. 11); 3 patients who presented with the same degree of reaction to both the patch test and the photopatch test (2 women and a 3-year-old girl); and a fifth patient who presented with photoaggravation (no. 14). The age of the octocrylene-positive patients (patch test and photopatch test) ranged from 3 to 56 years for females and from 24 to 71 years for men on the occasion of testing.

Of the 23 octocrylene-positive patients, 20 had presented intolerance reactions to sunscreens. According to patients' own statements, some of which referred to occurrences dating back as far as 15 years, 14 had previously used ketoprofen (Fastum®; Menarini, Florence, Italy) and 10 had suffered from a (severe) skin reaction to it (nos. 1, 2, 12–14, 18, 19, 21, 22, and 23). Six patients had never been exposed to ketoprofen, but had suffered complaints caused by sunscreen products. Three patients had presented with dermatitis caused by ketoprofen, but had no known sunscreen intolerance (nos. 9, 15, and 20).

In the cohort of 23 octocrylene-positive patients, there were 17 patients who had a history of use of, or intolerance to, topical ketoprofen. These 17 patients were photopatch tested with both ketoprofen and octocrylene, and all showed positive photopatch reactions to both compounds (Table 1). Two of them had presented with a positive patch test reaction to ketoprofen, but these reactions increased in severity after irradiation. Fifteen of these 17 patients were also recorded as having a positive photopatch test reaction to benzophenone-3. A photograph of one of the patients with positive photopatch reactions to octocrylene, ketoprofen and benzophenone-3 is given

¹ Sun exposure had not influenced the skin complaints, so octocrylene was not photopatch tested. However, to find other relevant allergies, the patient was photopatch tested with other sunscreen agents.

**Fig. 2.** Photograph of a patient with positive photopatch reactions to benzophenone-3, octocrylene, and ketoprofen.

in Fig. 2. Five patients with intolerance to sunscreens, without previous exposure to ketoprofen but with a positive photopatch test reaction to octocrylene (4 of these were not photopatch tested with ketoprofen), reacted negatively to benzophenone-3.

Skin-sensitizing potency

The sensitizing potency of octocrylene as assessed in the murine LLNA is shown in Table 2 and Fig. 3. It was shown to be a moderate sensitizer, with an EC3 value of 7.7% or 0.21 M.

Octocrylene chemical reactivity study

The chemical reactivity of octocrylene was investigated with three different nucleophiles: benzylamine, protected lysine (*N*_α-Ac-L-Lys-Me-ester), and protected cysteine (*N*-Ac-L-Cys-Me-ester). The lysine derivative was added to the reaction as a hydrochloric salt. Therefore, an equimolar amount of NaOH was added to this reaction mixture to neutralize the hydrochloric acid. The solvent system used in the reactivity experiments with benzylamine and lysine was ACN/carbonate buffer pH 10.0 (3:1). The reason for using a buffer of pH 10.0 is that a basic pH is commonly used in chemical reactivity experiments for detecting contact allergens reacting with lysine (21, 22). Benzylamine and the protected lysine reacted with octocrylene with the same reaction rates ($k = 0.055 \text{ hr}^{-1}$, assuming

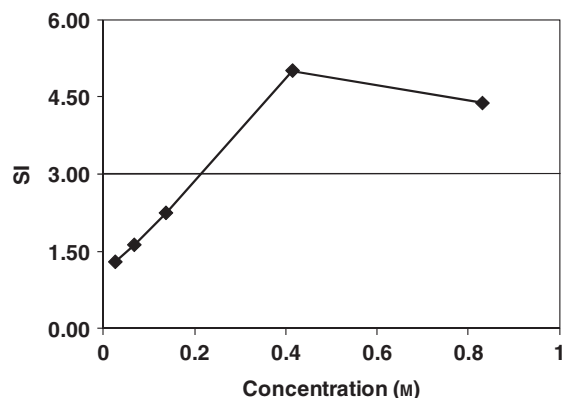


Fig. 3. Dose–response curve for octocrylene tested in the local lymph node assay. The concentrations are given in moles per litre. The horizontal line marks a stimulation index of 3, the cutoff limit for a compound to be considered a sensitizer.

pseudo-first-order kinetics; Fig. 4). When the reactivity of benzylamine towards octocrylene was investigated in EtOH, to determine whether the solvent had any influence on the reaction rate, the reaction rate was twice as high in EtOH ($k = 0.109 \text{ hr}^{-1}$) as in the ACN/carbonate buffer pH 10.0 (3:1) (Fig. 4). Thus, the solvent seems to have an impact on the reaction rate. The protected lysine was not soluble in EtOH, therefore no studies in EtOH could be performed with this nucleophile. It is noteworthy that benzylamine showed the same reactivity as the protected lysine derivative, which is more expensive and less soluble, in the octocrylene reactivity study. In analogy with the suggestion by Chipinda et al. (23) to use nitrobenzenethiol as a substitute for cysteine, benzylamine seems to be a candidate as a primary amine nucleophile modelling protein reactivity in simple chemical assays, as a complement to the more biomimetic peptide-based assays.

According to LC/MS, the major products formed in the octocrylene reactivity study were benzophenone and the corresponding imines. The structures of the different nucleophiles and the major products for the two reaction systems are shown in Fig. 5. To verify these results, reference compounds of the imines and benzophenone were used. Benzophenone-*N*-benzylimine was purified from the reactivity experiment in EtOH, benzophenone-*N*_α-Ac-L-Lys-Me-ester-imine was synthesized, and benzophenone was commercially available. To exclude the possibility that benzophenone is formed from octocrylene itself when water is present in ACN/carbonate buffer pH 10.0 (3:1), the corresponding control experiment was performed. However, no degradation of octocrylene or formation of benzophenone could be seen, even after 21 hr (results can be found in S1 Supporting Information). Benzophenone

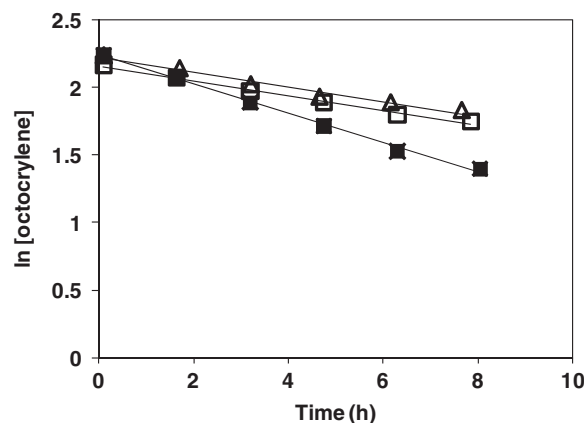


Fig. 4. Depletion of octocrylene with two different nucleophiles and two different solvent systems. The straight lines shown were obtained by least-squares linear regression of the logarithm of the octocrylene concentrations versus time. The slopes k correspond to the pseudo-first-order rate constants for the reactions: *N*_α-Ac-L-Lys-Me-ester in acetonitrile (ACN)/carbonate buffer pH 10.0 (3:1), $k = 0.055 \text{ hr}^{-1}$ ($r^2 = 0.973$) (Δ); benzylamine in ACN/carbonate buffer pH 10.0 (3:1), $k = 0.055 \text{ hr}^{-1}$ ($r^2 = 0.992$) (□); and benzylamine in ethanol (EtOH), $k = 0.109 \text{ hr}^{-1}$ ($r^2 = 0.996$) (■). The initial concentrations of octocrylene and nucleophile were 10 and 100 mM, respectively. Reactions were analysed by high-performance liquid chromatography (HPLC)/mass spectrometry and quantified by HPLC/ultraviolet spectroscopy with standard curves for octocrylene and the internal standard (dibutylphthalate).

is formed when the imine is hydrolysed by water, and an equilibrium is established between the imine and the corresponding amine and benzophenone. A tentative mechanism for octocrylene's reaction with amines is shown in Fig. 6.

The experiment on the reactivity of octocrylene towards protected cysteine was performed in EtOH, as the rate of the reaction between benzylamine and octocrylene was higher in EtOH than in ACN/carbonate buffer pH 10.0 (3:1). However, no reaction products could be seen even after 9 days (results can be found in S1 Supporting Information). Cysteine is expected to oxidize over time, and even if the cysteine dimer was detected, free cysteine was still the most abundant form of cysteine after 9 days.

Discussion

Photocontact allergy to octocrylene

Ketoprofen is a well-known photoallergen (11, 13, 24). Interestingly, all patients in this study who had used or been photosensitized to ketoprofen also presented with positive photopatch test reactions to octocrylene, and the

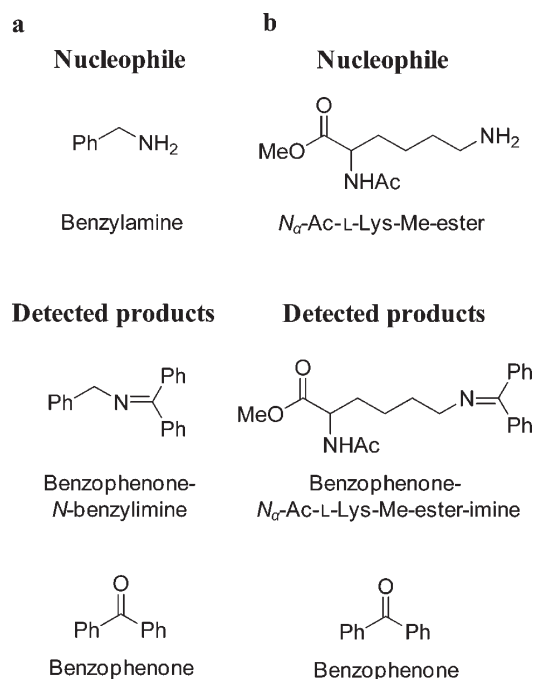


Fig. 5. The two different nucleophiles used in the chemical reactivity experiments with octocrylene, and the products detected for the two systems. (a) Structure of the nucleophile and detected products when benzylamine was used as nucleophile. (b) Structure of the nucleophile and detected products when *N*_α-Ac-L-Lys-Me-ester was used as nucleophile.

majority of them also reacted to benzophenone-3. This is in agreement with earlier findings in the literature (11, 15); for example, in the recent case series by Avenel-Audran et al. (15), a very high concordance (80%) of positive photopatch test reactions to ketoprofen and octocrylene was seen. In the current study, 6 patients with positive test reactions to octocrylene stated that they had not used ketoprofen. Notably, none of these patients reacted to benzophenone-3. Unfortunately, these subjects were not photopatch tested with ketoprofen, except for 1 patient, who, interestingly, did not react to it.

Apparently, ketoprofen leads to photosensitivity towards octocrylene, and, in the majority of cases, also to benzophenone-3. Whether the opposite is also true needs to be verified in larger studies in which octocrylene, ketoprofen and benzophenone-3 are photopatch tested simultaneously. Unfortunately, this was not performed at all times in the present clinical study.

Although photocontact allergy is the most frequently observed adverse reaction to octocrylene, it is obvious from the clinical studies that octocrylene also causes contact allergy. Up to now, no studies have been reported that have identified the chemical properties

responsible for either of these adverse effects. To unravel the underlying mechanisms involved in photocontact allergy – in general, a much less explored area than contact allergy – a thorough understanding of the corresponding mechanism for the origin of contact allergy would be instrumental, not least to distinguish between the two effects. A mechanistic model to explain the potency of octocrylene as a contact allergen is, of course, also important for predictive, diagnostic and prevention purposes. In the remainder of the article, we focus on the potency of octocrylene as a contact allergen and the mechanistic origin of this effect.

Contact allergy to octocrylene

Octocrylene was classified (19) as a moderate allergen on the basis of the LLNA experiment. Considering that octocrylene is approved by the EU (6) and FDA (7) in concentrations as high as 10% in sunscreens and cosmetic preparations, it is likely that people could be sensitized to octocrylene, without activation by UV radiation. This is supported by the results from the clinical study, which show that about one-fifth of the octocrylene-positive patients react without UV radiation. This is further supported by other recent clinical studies, which have reported that a significant number of patients have positive patch reactions to octocrylene (9, 10, 13, 15). In these studies, the proportion of octocrylene-positive patients reacting without UV radiation was 35–40%, which is even more than in the present study. It is noteworthy that, in children, contact allergy to octocrylene is much more common than photocontact allergy (9, 10, 15). Also, in the current clinical study, 2 of the 3 children had positive patch test reactions to octocrylene.

The chemical experiments show that octocrylene reacts with amines (Fig. 6), which, in the body, are mainly found in the form of lysine residues. Octocrylene reacts with benzylamine and lysine at room temperature via initial Michael-type addition followed by a reaction sequence corresponding to a retro-aldol reaction. Thus, it is likely that the Michael acceptor property is the reason for the allergenic activity of octocrylene in the absence of UV radiation. Although this type of retro-aldol condensation reaction has been observed before (25, 26), it has not previously been suggested as a mechanism in the formation of immunogenic hapten–protein complexes. The mechanism is closely related to the Schiff base mechanism, with the difference that the amine is attacking the conjugated β -carbon instead of the carbonyl carbon. This mechanism makes octocrylene specific for amines such as lysine, because it

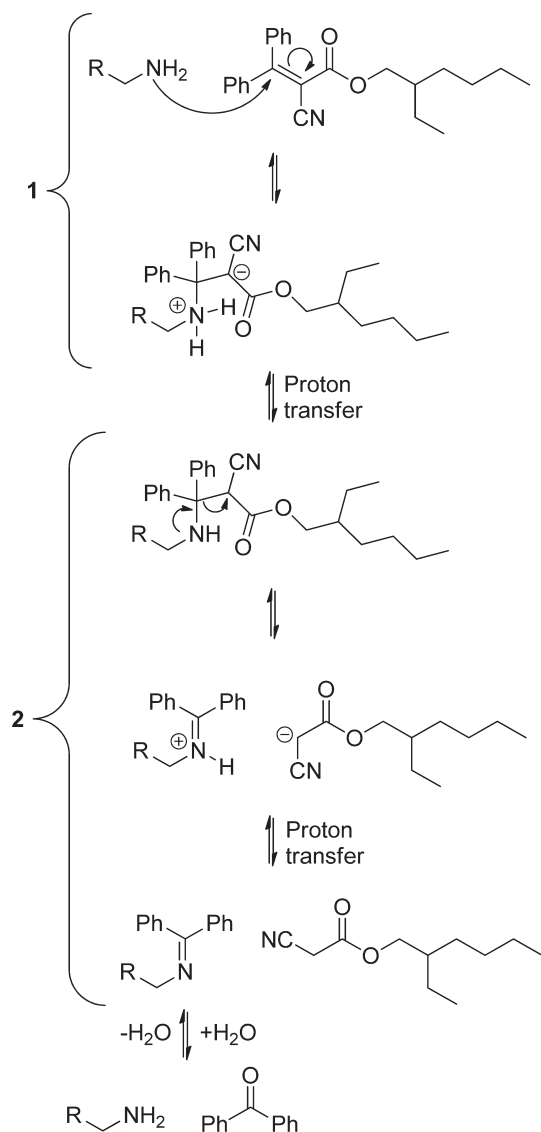


Fig. 6. Tentative mechanism for the formation of imine adducts when octocrylene reacts with amines. The first part (**1**) is a Michael addition and the second part (**2**) is a retro-aldol reaction with concomitant Schiff base formation.

will not form stable products with thiols such as cysteine, which is a sulfur nucleophile present in the body and that is often suggested to be involved in the formation of immunogenic hapten–protein complexes (21, 27). This hypothesis was verified by the chemical reactivity experiment with octocrylene and protected cysteine, in which no reaction products could be detected.

Interestingly, octocrylene is an α,β -unsaturated carbonyl compound, and this class of compounds is considered to form immunogenic hapten–protein complexes with thiols such as cysteine via a Michael

addition (28–31). In contrast to what was seen in our study, Nilsson et al. (29) showed that, when the hapten carvone (an α,β -unsaturated ketone) reacts with an amine, the nucleophile preferentially attacks the carbonyl carbon, forming an imine. This mechanism is called Schiff base formation, and is often considered when aldehydes form immunogenic hapten–protein complexes (32–34). However, Schiff base formation of α,β -unsaturated carbonyl compounds with amines is usually significantly slower than the Michael addition of thiols (29, 35, 36).

Although octocrylene behaves as a Michael acceptor in the initial steps of the reaction sequence with amines, the reaction outcome is not a Michael addition. Because of this, octocrylene cannot be classified according to the current structural alert-based classification system used to identify allergens (34, 35, 37). If classified according to the α,β -unsaturated carbonyl structural moiety, it would have been assigned to an erroneous mechanistic domain for skin sensitizers, that is, Michael-type nucleophilic addition. Octocrylene's reactivity towards amines and thiols demonstrates the complexity of predicting the bio-related reactivity of a compound. It shows the importance of using different nucleophiles (different amino acids or amino acid analogues) in reactivity experiments for screening of contact allergens. For example, the assay with glutathione, presented by Schultz et al. (30, 31), to predict the reactivity of α,β -unsaturated carbonyl compounds would not show any reactivity for octocrylene. As glutathione includes both a primary amine and a thiol, octocrylene could, in principle, react with the amine of glutathione. However, because only the amount of free thiol is monitored, this reaction would go undetected in the assay.

To summarize, the clinical studies show that octocrylene is both a contact allergen and a photocontact allergen. Although the present study does not offer any rationalization for the photosensitizing potency of octocrylene, we can make important conclusions regarding its ability to cause contact allergy. According to the LLNA, octocrylene is a moderate sensitizer, and it reacts with nucleophiles in a retro-aldol condensation reaction, a mechanism that has not previously been suggested to be involved in hapten–protein interactions. This mechanism makes octocrylene specific for amines such as lysine, whereas no stable reaction products could be observed for thiols such as cysteine. This is noteworthy because octocrylene is an α,β -unsaturated carbonyl compound, and these compounds are considered to be more reactive towards thiols than amines. We suggest that octocrylene's

reactivity towards amines is the explanation for its ability to cause contact allergy. Further reactivity studies, in the presence of UV light, are needed to reach the same level of understanding of its potency as a photocontact allergen.

Acknowledgements

We thank Professor Ann-Therese Karlberg at Dermatochemistry and Skin Allergy, Department of Chemistry, University of Gothenburg for valuable discussions and reading of the manuscript. The skilful technical assistance of Anders Eliasson and Susanne Exing at Experimental Biomedicine, Sahlgrenska Academy, University of Gothenburg is gratefully acknowledged. This work was performed within the Centre for Skin Research at University of Gothenburg, and was financially supported by the Swedish Research Council.

References

- Matsumura Y, Ananthaswamy H N. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol* 2004; **195**: 298–308.
- Scalia S, Mezzena M. Incorporation in lipid microparticles of the UVA filter, butyl methoxydibenzoylmethane combined with the UVB filter, octocrylene: effect on photostability. *AAPS PharmSciTech* 2009; **10**: 384–390.
- Duracher L, Blasco L, Jaoued A A, Vian L, Marti-Mestres G. Irradiation of skin and contrasting effects on absorption of hydrophilic and lipophilic compounds. *Photochem Photobiol* 2009; **85**: 1459–1467.
- Maier T, Korting H C. Sunscreens – which and what for? *Skin Pharmacol Physiol* 2005; **18**: 253–262.
- Damiani E, Rosati L, Castagna R, Carloni P, Greci L. Changes in ultraviolet absorbance and hence in protective efficacy against lipid peroxidation of organic sunscreens after UVA irradiation. *Photochem Photobiol B Biol* 2006; **82**: 204–213.
- European Commission. EU Council Directive of the EC (76/768/EEC). Annex VII: List of permitted UV filters which cosmetic products may contain, 2008: 121–126.
- Food and Drug Administration DOH and HS. Sunscreen drug products for over-the-counter human use: final monograph. *Fed Regist* 1999; **64**: 27666–27693.
- Carrotte-Lefebvre I, Bonneville A, Segard M, Delaporte E, Thomas P. Contact allergy to octocrylene – first 2 cases. *Contact Dermatitis* 2003; **48**: 46–47.
- Madan V, Beck M H. Contact allergy to octocrylene in sunscreen with recurrence from passive transfer of a cosmetic. *Contact Dermatitis* 2005; **53**: 241–242.
- Delplace D, Blondeel A. Octocrylene: really non-allergenic? *Contact Dermatitis* 2006; **54**: 295–295.
- Devleeschouwer V, Roelands R, Garmyn M, Goossens A. Allergic and photoallergic contact dermatitis from ketoprofen: results of (photo) patch testing and follow-up of 42 patients. *Contact Dermatitis* 2008; **58**: 159–166.
- Bennåsar A, Grimalt R, Romaguera C, Vilaplana J. Two cases of photocontact allergy to the new sun filter octocrylene. *Dermatol Online J* 2009; **15**: 14.
- Pigatto P D, Guzzi G, Schena D et al. Photopatch tests: an Italian multicentre study from 2004 to 2006. *Contact Dermatitis* 2008; **59**: 103–108.
- Vigan M. REVIDAL-GERDA: organisation and collaboration with pharmacovigilance. *Therapie* 2002; **57**: 263–264.
- Avenel-Audran M, Dutartre H, Goossens A et al. Octocrylene, an emerging photoallergen. *Arch Dermatol* 2010; **146**: 753–757.
- Wahlberg J E, Lindberg M. Patch testing. In: *Contact Dermatitis*, 4th edition, Frosch P J, Menné T, Lepoittevin J-P (eds): Berlin, Heidelberg, New York, Springer, 2006: pp. 365–390.
- Gerberick G F, Ryan C A, Dearman R J, Kimber I. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. *Methods* 2007; **41**: 54–60.
- Basketter D, Lea L, Dickens A, Briggs D, Pate I, Dearman R, Kimber I. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxicol* 1999; **19**: 261–266.
- Kimber I, Basketter D, Butler M et al. Classification of contact allergens according to potency: proposals. *Food Chem Toxicol* 2003; **41**: 1799–1809.
- Cainelli G, Giacomini D, Trere A, Boyle P P. Efficient transamination under mild conditions: preparation of primary amine derivatives from carbonyl compounds via imine isomerization with catalytic amounts of potassium tert-butoxide. *J Org Chem* 1996; **61**: 5134–5139.
- Gerberick G F, Vassallo J D, Bailey R E, Chaney J G, Morrall S W, Lepoittevin J P. Development of a peptide reactivity assay for screening contact allergens. *Toxicol Sci* 2004; **81**: 332–343.
- Gerberick G F, Vassallo J D, Foertsch L M, Price B B, Chaney J G, Lepoittevin J P. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree lmodel approach. *Toxicol Sci* 2007; **97**: 417–427.
- Chipinda I, Ajibola R O, Morakinyo M K, Ruwona T B, Simoyi R H, Siegel P D. Rapid and simple kinetics screening assay for electrophilic dermal sensitizers using nitrobenzenethiol. *Chem Res Toxicol* 2010; **23**: 918–925.
- Hindsén M, Zimerson E, Bruze M. Photoallergic contact dermatitis from

Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Standard curves used for quantification of octocrylene in the chemical reactivity study, results from the stability study on octocrylene in ACN/carbonate buffer pH 10.0 (3:1) and results from the reactivity experiment with octocrylene and *N*-Ac-L-Cys-Me-ester.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

- ketoprofen in southern Sweden. *Contact Dermatitis* 2006; **54**: 150–157.
- 25 Wolken W A, Ten Have R, Van Der Werf M J. 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* 2000; **48**: 5401–5405.
 - 26 Wolken W A M, Tramper J, Van Der Werf M J. Amino acid-catalysed retroaldol condensation: the production of natural benzaldehyde and other flavour compounds. *Flavour Fragr J* 2004; **19**: 115–120.
 - 27 Divkovic M, Pease C K, Gerberick G F, Basketter D A. Hapten–protein binding: from theory to practical application in the in vitro prediction of skin sensitization. *Contact Dermatitis* 2005; **53**: 189–200.
 - 28 Ahlfors S R, Sterner O, Hansson C. 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* 2003; **16**: 59–68.
 - 29 Nilsson A M, Gäfvert E, Salvador L, Luthman K, Bruze M, Gruvberger B, Nilsson J L G, Karlberg A T. Mechanism of the antigen formation of carvone and related alpha,beta-unsaturated ketones. *Contact Dermatitis* 2001; **44**: 347–356.
 - 30 Schultz T W, Yarbrough J W, Johnson E L. Structure–activity relationships for reactivity of carbonyl-containing compounds with glutathione. *SAR QSAR Environ Res* 2005; **16**: 313–322.
 - 31 Schultz T W, Yarbrough J W, Hunter R S, Aptula A O. Verification of the structural alerts for Michael acceptors. *Chem Res Toxicol* 2007; **20**: 1359–1363.
 - 32 Roberts D W, Aptula A O, Patlewicz G. Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the Schiff base applicability domain for skin sensitization. *Chem Res Toxicol* 2006; **19**: 1228–1233.
 - 33 Roberts D W, Aptula A O, Patlewicz G. Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem Res Toxicol* 2007; **20**: 44–60.
 - 34 Enoch S J, Cronin M T D, Schultz T W, Madden J C. Quantitative and mechanistic read across for predicting the skin sensitization potential of alkenes acting via Michael addition. *Chem Res Toxicol* 2008; **21**: 513–520.
 - 35 Estrada E, Patlewicz G, Gutierrez Y. 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* 2004; **44**: 688–698.
 - 36 Cai J, Bhatnagar A, Pierce W M. Protein modification by acrolein: formation and stability of cysteine adducts. *Chem Res Toxicol* 2009; **22**: 708–716.
 - 37 Lepoittevin J P. Molecular aspects of allergic contact dermatitis. In: *Contact Dermatitis*, 4th edition, Frosch P J, Menné T, Lepoittevin J P (eds): Berlin, Heidelberg, New York, Springer, 2006: pp. 45–68.

SUPPORTING INFORMATION

Clinical and experimental studies of octocrylene's allergenic potency

Isabella Karlsson¹, Katrien Vanden Broecke², Jerker Mårtensson³, An Goossens², and Anna Börje^{*,1}

¹*Dermatochemistry and Skin Allergy, Department of Chemistry, University of Gothenburg, Gothenburg, Sweden*

²*Department of Dermatology, University Hospital Saint-Raphaël, Katholieke Universiteit, Leuven, Belgium*

³*Department of Chemical and Biological Engineering/Organic Chemistry, Chalmers University of Technology, Gothenburg, Sweden*

** To whom correspondence should be addressed:*

*Ass. Prof. Anna Börje
Dermatochemistry and Skin Allergy
Department of Chemistry
University of Gothenburg
Kemiv. 10
412 96 Gothenburg
Sweden
Tel: +46 31 786 9012
Fax: +46 31 772 3840
E-mail: aborje@chem.gu.se.*

Table of Contents

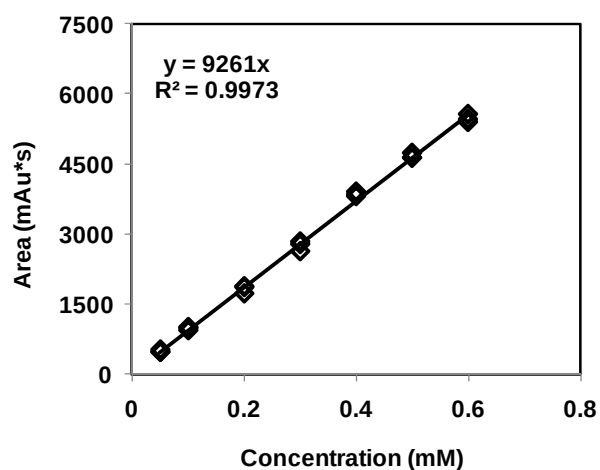
Standard curves for octocrylene and dibutylftalate.....	S3
Standard curves used for the reactivity experiments with benzylamine	S3
Standard curve for octocrylene at 305 nm	S3
Standard curve for dibutylftalate at 254 nm	S3
Standard curves for used for the reactivity experiments with N _α -Ac-L-Lys-Me-ester	S4
Standard curve for octocrylene at 305 nm	S4
Standard curve for dibutylftalate at 254 nm	S4
Results from stability study of octocrylene in ACN/carbonate buffer pH 10.0 (3:1)	S5
Results from reactivity study of octocrylene towards N-Ac-L-Cys-Me-ester	S5

Standard curves for octocrylene and dibutylftalate

Standard curves used for the reactivity experiments with benzylamine

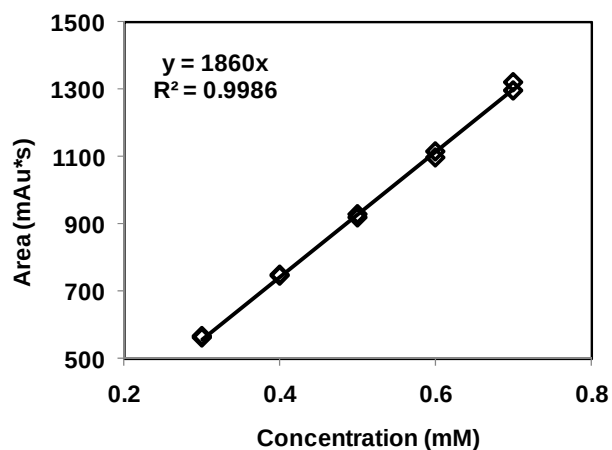
Standard curve for octocrylene at 305 nm

Conc. (mM)	Area(mAu*s)
0.05	472.95
0.05	476.16
0.05	522.40
0.1	933.22
0.1	981.68
0.1	1003.0
0.2	1722.0
0.2	1874.5
0.2	1856.7
0.3	2622.1
0.3	2831.5
0.3	2774.5
0.4	3896.3
0.4	3799.7
0.4	3838.4
0.5	4619.9
0.5	4623.6
0.5	4725.0
0.6	5553.6
0.6	5385.2
0.6	5445.0



Standard curve for dibutylftalate at 254 nm

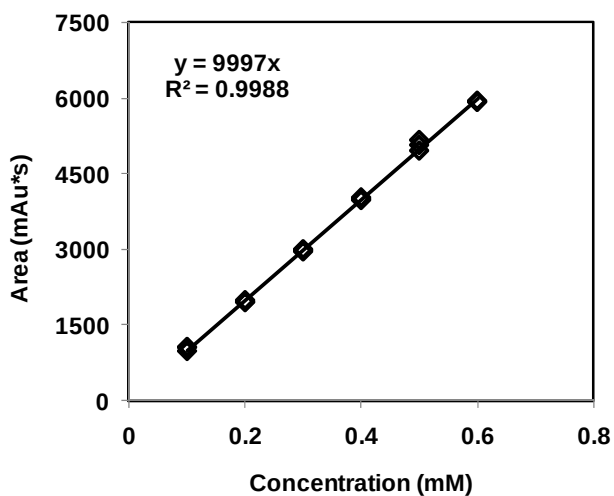
Conc. (mM)	Area (mAu*s)
0.3	566.81
0.3	562.53
0.4	746.30
0.4	749.12
0.5	928.76
0.5	919.28
0.6	1115.3
0.6	1097.4
0.7	1296.5
0.7	1321.0



Standard curves for used for the reactivity experiments with N_α -Ac-L-Lys-Me-ester

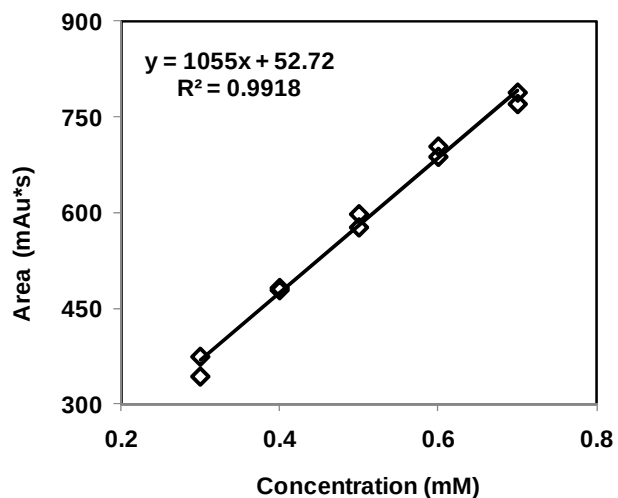
Standard curve for octocrylene at 305 nm

Conc. (mM)	Area (mAu*s)
0.1	1064.8
0.1	1057.6
0.1	984.72
0.2	1953.8
0.2	1960.4
0.2	1993.5
0.3	2985.5
0.3	2963.4
0.3	3004.3
0.4	3982.8
0.4	4008.2
0.4	4035.7
0.5	4964.5
0.5	5174.4
0.5	5076.7
0.6	5937.1
0.6	5932.8
0.6	5955.4



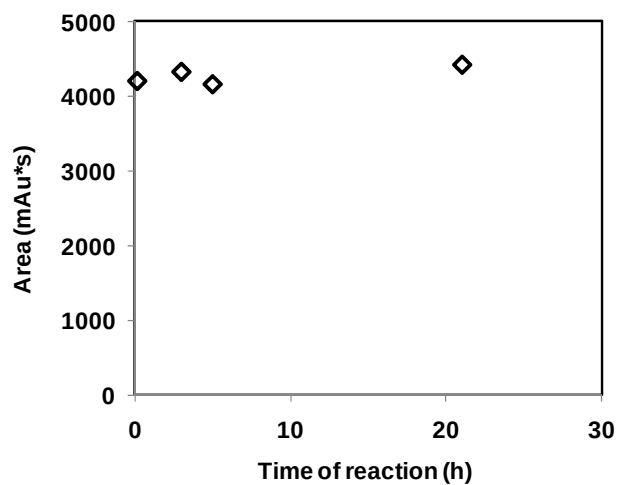
Standard curve for dibutylfthalate at 254 nm

Conc. (mM)	Area (mAu*s)
0.3	374.50
0.3	343.59
0.4	482.19
0.4	478.74
0.5	577.07
0.5	597.66
0.6	687.50
0.6	703.59
0.7	788.06
0.7	770.12



Results from stability study of octocrylene in ACN/carbonate buffer pH 10.0 (3:1)

Time (h)	Area (mAu*s)
0.17	4196.84
3.0	4319.47
5.0	4153.88
21	4414.78



Results from reactivity study of octocrylene towards *N*-Ac-L-Cys-Me-ester

Time (h)	Area (mAu*s)
0.17	4196.84
2.0	4319.47
6.0	4153.88
216	4414.78

