

Epoxyalcohols: Bioactivation and Conjugation Required for Skin Sensitization

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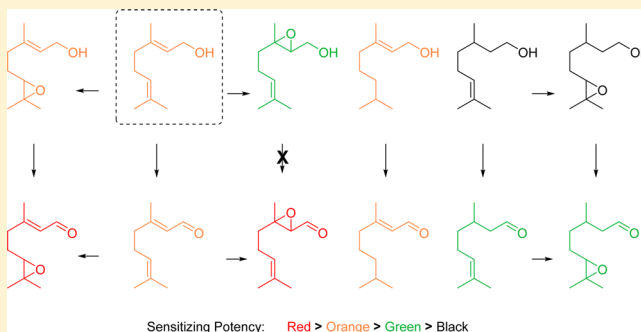
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Supporting Information

ABSTRACT: Allylic alcohols, such as geraniol **1**, are easily oxidized by varying mechanisms, including the formation of both 2,3-epoxides and/or aldehydes. These epoxides, aldehydes, and epoxy-aldehydes can be interconverted to each other, and the reactivity of them all must be considered when considering the sensitization potential of the parent allylic alcohol. An in-depth study of the possible metabolites and autoxidation products of allylic alcohols is described, covering the formation, interconversion, reactivity, and sensitizing potential thereof, using a combination of *in vivo*, *in vitro*, *in chemico*, and *in silico* methods. This multimodal study, using the integration of diverse techniques to investigate the sensitization potential of a molecule, allows the identification of potential candidate(s) for the true culprit(s) in allergic responses to allylic alcohols. Overall, the sensitization potential of the investigated epoxyalcohols and unsaturated alcohols was found to derive from metabolic oxidation to the more potent aldehyde where possible. Where this is less likely, the compound remains weakly or nonsensitizing. Metabolic activation of a double bond to form a nonconjugated, nonterminal epoxide moiety is not enough to turn a nonsensitizing alcohol into a sensitizer, as such epoxides have low reactivity and low sensitizing potency. In addition, even an allylic 2,3-epoxide moiety is not necessarily a potent sensitizer, as shown for **2**, where formation of the epoxide weakens the sensitization potential.



INTRODUCTION

Whether a particular chemical will be a skin sensitizer causing allergic contact dermatitis at repeated skin contact depends in large part on its ability to react with appropriate proteins in the skin. The correlation between reactivity and sensitization was initially demonstrated by Landsteiner and Jacobs in 1936.^{1,2} The ability to predict the sensitizing potency of a compound is thus dependent on the correctness in predicting its reactivity to skin proteins. This is the basis of structure–activity relationship (SAR) analysis for skin sensitization. The cornerstone of such predictions is most often the recognition of features (referred to as structural alerts) in the chemical structure that are associated with reactivity based on well-established principles of mechanistic organic chemistry. However, no truly predictive investigation into the chemical basis of skin sensitizing potency can be established without access to carefully evaluated experimental data from well-designed series of compounds. Similarity within the designed series regarding chemical structure, molecular weight, and hydrophobicity is important in order to, as accurately as possible, allow comparison of

allergic potency in relation to structure alone and to minimize variation due to other effects.

As skin sensitization from chemicals in our environment is an important health problem, it is of utmost importance to predict the sensitization potential of a new chemical for use in consumer products before it is introduced to the market. Prediction of the sensitization potential of compounds that need to be activated via different mechanisms to be able to react with skin proteins to form immunogenic complexes is more complex compared to that of compounds that can directly form immunogens without activation (haptens). Mainly, two different mechanisms for activation have been identified: activation outside the skin via autoxidation (prehaptens) or in the skin via bioactivation (prohaptens).³ Compounds containing double bonds that are susceptible to oxidation by autoxidation or by bioactivation can be activated both outside and inside the skin.^{4–10} With increased regulatory constraints

Received: July 17, 2014

for *in vivo* testing on animals, bioactivation in particular offers challenges for modelers and toxicologists. It is therefore important to perform multiple investigations or (Q)SAR studies using different methods for modeling the bioactivation.

Epoxidation is described as an important bioactivation route, and a double bond is the activation alert moiety, as metabolic oxidation occurs via enzymatically catalyzed oxidation of the double bond.^{11,12} In a previous study, we explored the reactivity and sensitizing potency of a carefully designed series of aldehydes and epoxyaldehydes (Figure 1, compounds 7–12).¹³ The chemical reactivity of these aldehydes toward a model hexapeptide was investigated by comparing the depletion of free peptide and identification of formed adducts.¹³ Experimental sensitization studies according to the murine local lymph node assay (LLNA)^{14,15} were performed to investigate their sensitizing potency. The results from the reactivity experiments were congruent with the classification of sensitizing potency (Table 2, compounds 7–12). The compounds differed by a factor of ca. 50 in sensitizing potency, confirming that even small changes in the chemical structure result in significant differences in sensitizing potency. Our data showed that the highly reactive 2,3-unsaturated aldehydes and 2,3-epoxyaldehydes are sensitizing moieties and should be considered as structural alerts; thus, the importance not only of double bond conjugation to aldehyde but also of activation via 2,3-epoxyaldehyde formation was demonstrated. Furthermore, observations indicated that the formation of nonactivated epoxides by bioactivation is not necessarily sufficient to significantly increase the potency of a weakly sensitizing parent compound.¹³

The aim of the present study was to further investigate the impact of epoxidation on the sensitizing potency of a compound, in particular to understand when epoxidation can turn a nonsensitizing compound into a sensitizer. Aliphatic alcohols are not considered, of themselves, reactive enough to cause sensitization. Therefore, the present investigations were undertaken using a series of alcohols (Figure 1, structures 1–6) corresponding to the previously studied aldehydes (Figure 1, structures 7–12) to allow comparisons especially with regard to bioactivation.

The investigated alcohols were designed to contain epoxides and/or double bonds, either conjugated to the alcohol moiety or not, in order to investigate whether epoxidation at different places in the structure will have an impact on the sensitizing potency of the compound. The studied epoxides are similar to those discovered by autooxidation.^{5,16} Various approved predictive testing techniques, encompassing *in vivo* (LLNA),^{14,15} *in vitro* (activation of keratinocytes according to the KeratinoSens^{17,18} model), *in chemico* (peptide reactivity), and *in silico* (density-functional models of reactivity) methods, were applied to obtain a thorough analysis of the structure–activity relationships including bioactivation.

EXPERIMENTAL PROCEDURES

Caution: These chemicals are dangerous. This study involves skin sensitizing compounds, which should be handled with particular care.

Instrumentation and Mode of Analysis. Electron–ionization mass spectral analysis (70 eV) was performed on a Hewlett-Packard 5973 mass spectrometer connected to a gas chromatograph (Hewlett-Packard 6890). The GC was equipped with a splitless capillary inlet and an HP-5MSi fused silica capillary column (30 m × 0.25 mm i.d., particle size, 0.25 μ m, Agilent Technologies, Palo Alto, CA, USA). Helium was used as carrier gas, and the flow rate was 1.1 mL/min. The inlet temperature was set at 250 °C. The temperature program started

at 35 °C for 1 min, increased by 10 °C/min, and ended at 250 °C for 5 min. For mass spectral analysis, the mass spectrometer was used in scan mode detecting ions with m/z values ranging from 50 to 1500.

LC/MS analyses were performed using electrospray ionization (ESI) on a Hewlett-Packard 1100 HPLC/MS. 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 HPLC was equipped with a HyPURITY C18 column (150 × 3 mm i.d., particle size 3 μ m, Thermo Hypersil-Keystone, Thermo Electron Corp., Bellafonte, PA, USA). The mobile phase consisted of 0.005% pentafluoropropanoic acid, 0.1% acetic acid, and 5% acetonitrile in water (solvent A) and 0.005% pentafluoropropanoic acid, 0.1% acetic acid, and 50% water in acetonitrile (solvent B). A linear gradient from 0 to 100% B in 20 min, followed by 10 min of isocratic elution at 100% B was used. The column was equilibrated with 0% B for 10 min between each run. Aliquots of 2 μ L were injected onto the column and eluted with a flow rate of 0.40 mL/min, and the column temperature was set to 40 °C. The ESI interface was used in positive ionization mode with the following spray chamber settings: nebulizer pressure, 40 psi; capillary voltage, 3500 V; drying gas temperature, 350 °C; and drying gas flow rate, 10 L/min. The fragmentor voltage was set to 120 V. The mass spectrometer was used in scan mode detecting molecular ions with m/z values ranging from 50 to 2000.

Chemistry. Geraniol (1) and the internal standard linalyl acetate (IS1) were purchased from Aldrich Chemicals (Stockholm, Sweden), 4, from Bedoukian Research, Inc. (Danbury, CT, USA), ($\pm$)-citronellol (5), from Sigma-Aldrich (Steinheim, Germany), and 1,2,3,5-tetramethylbenzene (IS2), from TCI Europe (Zwijndrecht, Belgium). 1, 4, 5, and IS1 were distilled prior to use. Ac-Pro-His-Cys-Lys-Arg-Met-OH (AcPHCKRM, 98%) was obtained from Peptide 2.0 Inc. (Chantilly, VA, USA). Acetone p.a. was purchased from Merck (Darmstadt, Germany), and olive oil, from Apoteket AB (Gothenburg, Sweden). Unless otherwise indicated, reagents were obtained from commercial suppliers and used without further purification. Column chromatography was performed using Merck silica gel Geduran Si 60 (0.063–0.200 mm). TLC was performed using silica gel coated aluminum plates (Merck, 60 F254) and developed with anisaldehyde dip (2.1 mL of acetic acid, 5.1 mL of anisaldehyde, and 7 mL of H₂SO₄ in 186 mL of ethanol) followed by heating. The purity of both synthesized and purchased test compounds was >99% (GC/MS) before testing of sensitization potential, reactivity toward model peptide, or metabolism. Compounds 2,¹⁹ 3,^{20,21} 6,²² and 13^{19–21} were synthesized as described in the literature.

Reactions of Compounds 1–6 toward the Model Peptide AcPHCKRM. All solvents were degassed with argon prior to use. Solutions of 1 in dimethyl sulfoxide (DMSO) (40 mM, 100 μ L) together with potassium phosphate buffer (100 mM, pH 7.4) (200 μ L) were added to a vial purged with argon containing AcPHCKRM in DMSO (4 mM, 100 μ L). Accordingly, final concentrations of 1 and the model peptide in the reaction mixture were 10 and 1 mM, respectively. The reaction was kept under argon at room temperature and was monitored with HPLC/UV_{210nm}-ESI-MS every 40 min for 24 h. Derivatives 2–6 were treated identically to 1, and their reactions were investigated using the method described above. The stability of the peptide AcPHCKRM, under the experimental conditions used, has previously been verified, and no dimerization or degradation was observed within 24 h.¹³ The stability of 1–6 was explored as described¹³ to exclude any risk of hydrolysis of the epoxides, but no degradation was observed for either of the derivatives within 24 h under the conditions of the reactivity experiment.

Computational Techniques. The P450 heme active site was modeled using a methoxy radical, as described in the literature.^{23–29} Reactivity calculations were carried out at the B3LYP-D3/6-31+G**^{30–35} level of theory in Jaguar,³⁶ part of the Schrödinger suite of programs. Structures were initially energy minimized in MacroModel³⁷ before an LST search was undertaken in Jaguar for the transition state. If no transition state was initially forthcoming, alternative attempts were tried, including variation of parameter QSTinit and the finding of initial *in vacuo* transition states that were

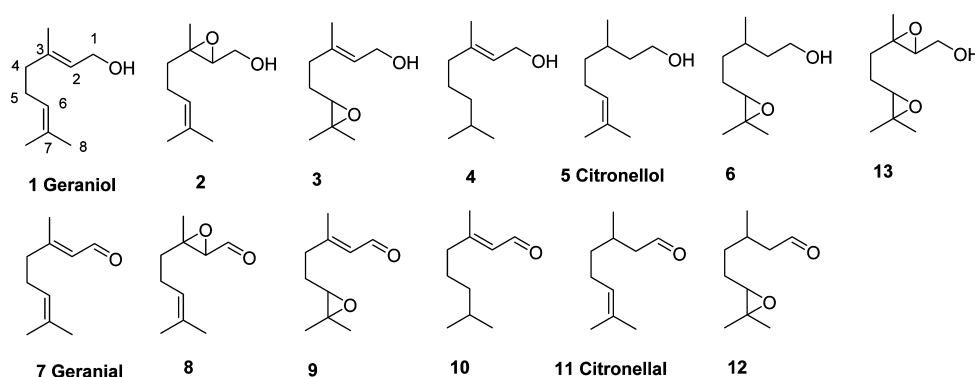


Figure 1. Structures of compounds 1–13. The epoxidation and reduction of double bonds in geraniol, 1, were studied. Compounds 2 and 3 contain epoxide moieties in the 2,3- and 6,7-positions, respectively. In 4 and 5, the double bonds are reduced in the 6,7- and 2,3-positions, respectively. In 6, a combination of reduction of the double bond in the 2,3-position and an epoxidation in the 6,7-position is introduced. Compounds 7–12 are analogous derivatives of geraniol, 7. Structure 13 is the possible diepoxide metabolite of 1. The indicated atom numbering is used throughout this article.

subsequently solvated. Calculations of the C2–C7 (for numbering of atoms, see Figure 1) distance of 3 and 6 were additionally resubmitted with the M05-2X³⁸ functional. The solvent used for all calculations was implicit water, using the PBF model.^{39,40} Results were analyzed using the Quick Reaction Coordinate (QRC) method.⁴¹ Calculations were performed on the SNIC facilities of C3SE, located at Chalmers University of Technology, Gothenburg. Due to the flexibility of the second terpene unit (C5 onward) as well as the lack of effect on reactivity from the oxidation state of the C6–C7 double bond, the structures described herein were, for most calculations, truncated (denoted where appropriate as 1t, 2t, 3t, ..., as shown in Scheme 2) by treating the molecule from C4 onward as a methyl group (electronically sufficiently similar to the longer alkyl chain).

Experimental Animals. Female CBA/Ca mice, 8 or 9 weeks of age, were purchased from B&K Sollentuna (Sollentuna, Sweden). The mice were housed in HEPA filtered air flow cages and kept on standard laboratory diet and water *ad libitum*. The local ethics committee in Gothenburg approved the study.

Sensitization Potential of 4 in Mice. The LLNA⁴² was used to assess the sensitization potential of compound 4, using a slight modification to the validated protocol. Where the protocol^{14,15,42} uses three groups of five mice, and a further control group, the method employed (as previously published^{13,43} by the authors) uses five groups of three mice (exposed, respectively, to concentrations of 1, 5, 20, 40, and 80% w/w of 4 in 4:1 acetone/olive oil (AOO)) and a further control group of four mice exposed to vehicle alone. This enhances the predictive capacity of the assay by providing additional data points.

Stability Experiments of Epoxides 2, 3, 8, 9, and 13. Prior to performing the bioactivation experiments, the chemical stability of the epoxides 2, 3, 8, 9, and 13 to the incubation conditions (potassium phosphate buffer (100 mM, pH 7.4), 60 min, 37 °C) was investigated. Stock solutions of each epoxide (5 mM) in DMSO were prepared. The stability experiments were initiated by addition of the epoxide stock solution (1 μ L) to prewarmed potassium phosphate buffer (499 μ L, 100 mM, pH 7.4). The solution was either incubated at 37 °C for 60 min or extracted immediately without incubation. The extracts were analyzed with GC/MS using synthesized reference compounds. After 60 min incubation, 85% of 2, 90% of 3, 98% of 8, 72% of 9, and 79% of 13 remained.

Incubation of 2 and 3 with Human Liver Microsomes. The microsomal incubations were performed using human liver microsomes (HLM) (0.5 mg of protein, pooled from 29 male and female donors, BD Biosciences), substrate (10 μ M), potassium phosphate buffer (100 mM, pH 7.4), and a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system (1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium chloride, BD Biosciences) in a total volume of 500 μ L. All incubations were performed in triplicate, and control

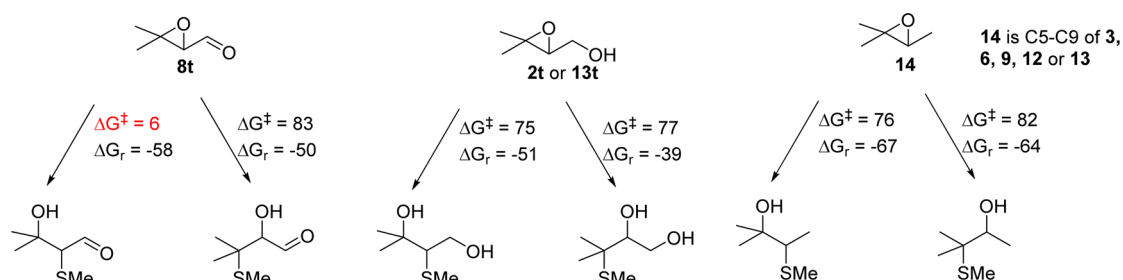
samples were run in the absence of substrate or in the absence of the NADPH regenerating system. The incubations were initialized by the addition of the substrate after 5 min of preincubation at 37 °C and were terminated after 60 min by addition of ethyl acetate (500 μ L) containing IS2 (2 μ M). The extracts were collected after centrifugation at 3000 rpm for 10 min at 4 °C and dried over 4 Å molecular sieves. IS1 was added to the solution prior to analysis. Analysis was performed using GC/MS.

GC/MS Analysis of 2 and Metabolites (2, 3, 8, 9, and 13). The analysis of the incubation mixtures was performed with the following method settings: the temperature program started at 55 °C for 3 min, increased by 5 °C/min, ending at 160 °C for 1 min, followed by an increase of 15 °C/min, ending at 250 °C for 5 min. The injection volume was 2 μ L. The mass spectrometer was used in selected ion monitoring (SIM) mode. Two methods with different *m/z* values were used. For the detection of potential metabolites of 2, the *m/z* values were monitored as follows: 199 and 134 at *t_R* = 5.00–13.15 min (IS2); 150, 109, and 69 at *t_R* = 13.15–15.65 min (8); 136, 121, and 93 at *t_R* = 15.65–17.00 min (IS1); 139, 82, and 69 at *t_R* = 17.00–19.00 min (2); and 111, 84, and 71 at *t_R* = 19.00–36.00 min (13). For the detection of the potential metabolites of 3, the *m/z* values were monitored as follows: 134 and 119 at *t_R* = 5.00–13.00 min (IS2); 136, 121, and 93 at *t_R* = 13.00–18.00 min (IS1); 153, 97, and 85 at *t_R* = 18.00–19.00 min (9); 152, 85, and 71 at *t_R* = 19.00–19.80 min (3); and 111, 84, and 71 at *t_R* = 19.80–36.00 min (13).

KeratinoSens Assay Procedure. The KeratinoSens assay uses KeratinoSens reporter keratinocytes, which contain a stable insertion of a luciferase gene under control of the ARE element of the AKR1C2 gene. The KeratinoSens assay was performed according to the standard operating procedure.^{18,44} Briefly, cells were grown for 24 h in 96-well plates. The medium was then replaced with medium containing the test compound and 1% DMSO. Each compound was tested at 12 binary dilutions in the range from 0.98 to 2000 μ M. In each independent repetition, three parallel replicate plates were run for luciferase determination, and a fourth parallel plate was prepared for cytotoxicity determination. Cells were incubated for 48 h with the investigated compounds, after which luciferase activity and cytotoxicity (with the MTT assay⁴⁵) were determined. For each compound, 5 or 6 independent repetitions were performed.

KeratinoSens Assay Procedure with S9 extract. The KeratinoSens assay in the presence of S9 extract was performed according to the published protocol for investigation of potential bioactivation.⁴⁶ Cells were prepared in 96-well plates as in the standard assay. For treatments without S9, a stock solution of 0.2 mM NADPH, 1 mM D-glucose-6-phosphate, and 1% FCS in clear DMEM was prepared. For treatments with S9, the same stock solution was prepared and further enriched with 4% of S9 fractions (LS9 SD Arcolor 1254, lyophilized, from male Sprague–Dawley rat liver, Trinova, Giessen, Germany). Both solutions were filtered (0.22 μ m

Scheme 1. $\Delta G^\ddagger$ and ΔG_r (all in kJ mol⁻¹) Derived in Jaguar at the B3LYP-D3/6-31+G** Level for Nucleophilic Opening of Epoxide Moieties with Varying Substituents^a



^a—CH=O, —CH₂OH, and —CH₃ substituents correspond to 8, 2, and 3, respectively. Structures 1t, 2t, ... indicate truncation of the terpene structure at C4 to speed calculations.

Table 1. Ions and Fragments Observed for the Monoadduct Formed from 6 in the Reactivity Experiment with AcPHCKRM^a

compd (MW)	adduct	[M* + H] ⁺ m/z	[M* + 2H] ²⁺ m/z	y _s * m/z	y ₄ * m/z	y ₃ m/z	y ₂ m/z	b ₂ m/z	b ₁ m/z
6 (172.3)		983.5	492.2	844.4	707.3	434.2	306.1	277.1	140.1

^aThe reactivity experiments were all performed in DMSO/potassium phosphate buffer at pH 7.4 (1:1) with a 10-fold excess of the hapten. The reactions were kept at room temperature and monitored with HPLC-UV-ESI-MS. M* or y* indicates haptenated peptide or haptenated fragments, respectively. The mass of the unhaptenated peptide is 813.2. The mass of the unhaptenated fragments are as follows: b₁, 140.1; b₂, 277.1; y₂, 306.2; y₃, 434.1; y₄, 537.3; y₅, 674.3. Fragment assignments can be found in the Supporting Information, Figure S1.

Millipore Express membrane). The test compounds dissolved in DMSO were added to the solutions with/without S9, and 50 μ L of these mixtures was added to the cells in different wells already containing 150 μ L of 1% FCS in transparent DMEM. Final DMSO and S9 concentrations were thus at 1%. In each experiment, each compound was tested in triplicate at each of the six concentrations. Four independent repetitions of the experiment were carried out. After 48 h incubation with the test chemicals, the medium was removed and replaced with 100 μ L of PrestoBlue reagent (Invitrogen, Zug, Switzerland) diluted 10-fold in transparent DMEM to determine cytotoxicity. Plates were incubated for 30 min at 37 °C and 5% CO₂. The fluorescence at 560 nm excitation and 590 nm emission was determined, and the plates were washed with 200 μ L of PBS. Cells were then lysed, and luciferase activity was determined.

RESULTS AND DISCUSSION

The sensitizing potencies of epoxyalcohols 2, 3, and 6 and of their corresponding unsaturated alcohols 1, 4, and 5 have been elucidated using various techniques including studies on bioactivation. Overall, the sensitization potential was found to derive from a metabolic oxidation to the more potent aldehyde (1, 3, and 4). In cases where oxidation is less likely (2) or the resulting aldehydes are less reactive (5 and 6), the compound remains weakly or nonsensitizing. Metabolic activation of a double bond to form a nonconjugated, nonterminal epoxide moiety was not enough to turn a nonsensitizing alcohol into a sensitizer. A complex pattern of possible reactivity was elucidated, also including the potential oxidation products 7–13. It was observed that formation of a 2,3-epoxide results in a large change in the behavior of a compound, decreasing the sensitizing potency of the alcohol while increasing that of the aldehyde, whereas the changes introduced by a 6,7-epoxide are less dramatic.

Design of Studied Derivatives. A series of six alcohols was chosen (1–6, Figure 1), representing all possible combinations of epoxidation or unsaturation of the two double bonds in 1. The series does not include diepoxide 13, which was synthesized as a reference and searched for in HLM incubations as a potential further metabolite of 2 or 3 but was not observed.¹³ Maximal similarity of chemical structure,

molecular weight, and hydrophobicity was desired in order to allow accurate comparisons of the effect of reactivity on allergenic potency while minimizing variation in other properties that could affect the LLNA response. The reduced forms, in particular derivatives 5 and 6, allow investigation of the sensitizing potency when neither activation of the alcohol by epoxidation nor the effects of the conjugated double bond are possible, whereas the epoxidized forms allow us to investigate directly the potency of the possible metabolites. Alcohols 1–6 are theoretically susceptible to oxidation of the alcohol into the corresponding aldehyde (forming the previously studied compounds 7–12).¹³

Reactivity of 1–6 toward the Model Peptide AcPHCKRM. To investigate the chemical reactivity of compounds 1–6, their reactions with the nucleophilic hexapeptide AcPHCKRM were analyzed at pH 7.4 using a 10-fold excess of test chemical in a mixture of phosphate buffer/DMSO. This peptide has previously been used to assess the reactivity of potential contact allergens.^{13,47} The N-acetylation was performed to exclude reactions at proline as previously described.^{48–50}

The percentage peptide depletion was calculated every 40 min during 24 h for 1–6, and no depletion was observed. As expected, the alcohol entities in 1–6 are nonreactive. Under our experimental conditions, neither the 2,3-epoxy moiety in 2 nor the 6,7-epoxy moiety in 3 and 6 was reactive enough to cause observable peptide depletion. Reasoning for this can be found in the results of reactivity calculations (DFT at the B3LYP-D3/6-31+G** level) shown in Scheme 1. Opening of either end of a 2,3-epoxy or 6,7-epoxy group in the absence of an activating aldehyde is a moderately high-energy pathway ($\Delta G^\ddagger$ of between 75 and 82 kJ mol⁻¹ for all combinations), whereas attack at the 2-position of a conjugated epoxy group (the moiety found in 8 only) is a very reactive process with a $\Delta G^\ddagger$ of a mere 6 kJ mol⁻¹. This corresponds to the easy opening and reactive behavior of the epoxy-aldehyde, as described both in this article (Table 2) and previous work.^{13,43} It is interesting to note the reversal, based on these calculations, of selectivity achieved by epoxidation of the double

bond: a 2,3-unsaturated aldehyde is typically held to be reactive toward soft nucleophiles at the 3-position, whereas the epoxy-aldehyde is much more reactive at the 2-position.

A cysteine adduct was detected after 200 min in reactivity experiments of the peptide and **6**, as indicated by the appearance of $[M^* + H]^+$ and $[M^* + 2H]^{2+}$ ions (* denotes one haptentation) in the mass spectrum (Table 1). In addition, signals corresponding to y^* , y^*_4 , y_3 , y_2 , b_2 , and b_1 fragments were observed (see Figure S1 in the Supporting Information for fragment assignments), showing that the cysteine residue of the peptide has been modified. The formation of small amounts of adduct in the absence of significant depletion of unreacted peptide has been reported previously.^{13,51,52,55,56} In our previous study, a similar adduct was detected after 200 min in the reaction of the peptide with **12**, but no significant peptide depletion was observed. As for the conjugate formed from **12** and the peptide, the adduct formed with **6** was possibly due to a ring opening of the epoxide on C6 by the cysteine. A potential reason for the ability of **6** to form a conjugate where **3** does not is stacking of the π -system of the double bond in **3** with the orbitals of the 6,7-epoxy group, which is not possible with **6** (Figure 2). A search across all conformers within 20 kJ mol⁻¹ of

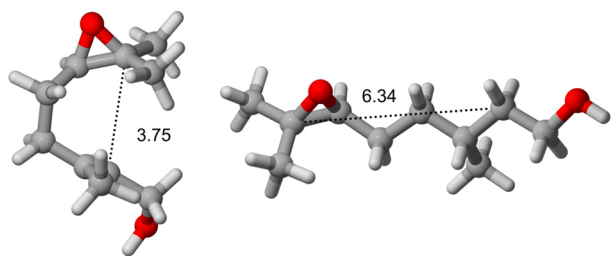


Figure 2. π -Stacking behavior of **3** (left), compared to a linear conformation of **6** (right). The figure shows the lowest-energy conformers of the two, as found by MacroModel; energies are derived in Jaguar at the M05-2X/6-31+G** level of theory. Distances are in ångströms.

the minimum was performed for both compounds at the M05-2X/6-31+G** level of theory.^{30–38} This resulted in a set of possible distances, each of which is attached an energy that can be converted via Boltzmann distribution into probabilities, resulting in statistical distributions of the distance. For **3**, this is $\mu = 5.19$ Å and $\sigma = 0.54$, and for **6**, this is $\mu = 5.80$ and $\sigma = 0.29$. The distance in **3** is therefore shorter, indicating a greater proportion of time spent in a folded structure and hence a lower availability of the epoxide to react with the peptide and thus a slower or negligible rate of reaction. In addition to the statistically shorter average C2–C7 distance, the 10 most folded conformers of **3** are all shorter than 4 Å. This includes the only two conformers within 2 kJ mol⁻¹ of global minimum, between them giving a probability of 15% by a Boltzmann distribution. However, no conformers of **6** fall below the 4 Å threshold, and the global minimum conformers of **6** occur at 6.34 and 5.40 Å.

In all reactions, a peptide dimer was observed in the reactivity experiments, as indicated with the appearance of ions at m/z 1624.5, 812.3, and 542.2. These ions correspond to $[M_{\text{peptide dimer}} + H]^+$, $[M_{\text{peptide dimer}} + 2H]^{2+}$, and $[M_{\text{peptide dimer}} + 3H]^{3+}$, respectively. In reactions with **2** and **3**, the dimer appears already after 40 min, with **1**, **4**, and **6**, after 80 min, but with **5**, only after 160 min. The ability of alcohols to catalyze dimer formation of the unreacted peptide containing cysteine

has been observed previously.^{51,53} However, in the present study, the dimer was formed both earlier and in greater quantity. The retention times of the dimer and the unreacted peptide were similar, so no ratio could be calculated, but unreacted peptide was still left according to the fragmentation in MS after 24 h in reactions with **1–6**. Thus, the formation of the dimer could not explain the lack of reactivity of epoxides **2**, **3**, and **6**.

Skin Sensitization Potency According to the LLNA.

The murine local lymph node assay (LLNA) was used to assess the skin sensitizing potency of compound **4**. The LLNA results for **1–3**, **5**, and **6** have been reported previously.^{5,6,22} LLNA results are expressed as EC₃ values, which is the estimated concentration of a compound required to induce a 3-fold increase in sensitization compared to a control.

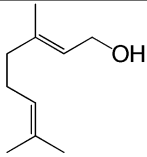
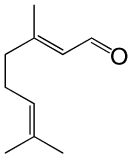
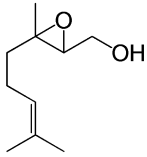
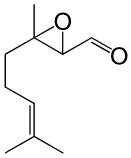
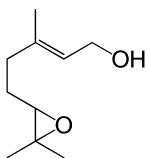
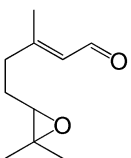
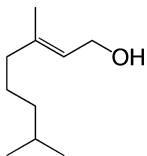
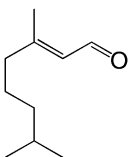
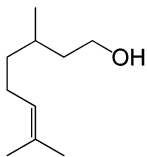
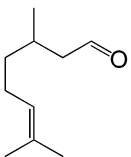
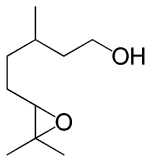
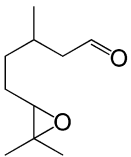
The EC₃ value obtained for **4** was 0.93 M (15% w/v) (Table 2, Figure 3, and detailed information in Table S1, Supporting Information). Compound **4** was expected to be a sensitizer (after activation to **10**), analogous to the results from the previously published sensitization studies of **1** (1.5 M)⁵ and **3** (0.42 M).⁶ The importance of the conjugation for the sensitizing potency was confirmed when no EC₃ values were obtained at the tested concentration (up to 5.3 and 4.7 M) for nonallylic alcohols **5** and **6**, respectively.²² Conjugated aldehydes, in general, are significantly stronger sensitizers than their related allylic alcohols. In the present study, a consistent increase in potency upon oxidizing the alcohol C–O bond in the three cases, **1** vs **7**, **3** vs **9**, and **4** vs **10**, was observed. This can be explained by considering the allylic alcohol itself as either a weak sensitizer or nonsensitizer, which is oxidized *in vivo* to the corresponding, much more strongly sensitizing, conjugated aldehydes. As the investigated alcohols differ only in the C6–C7 region, they are affected in a similar way by oxidation.

Incubation of 2 and 3 with Human Liver Microsomes (HLM). Incubations of **2** and **3** with HLM were carried out to explain the low sensitizing potency obtained for **2** in comparison to that for **1**, **3**, and **4**. In incubation of **2** with HLM, it could be expected that metabolites **8** and **13** were able to be formed, but no metabolites were detected (Figure 4), only the starting material **2** was recovered, suggesting a stability of **2** to microsomal activation. When **3** was incubated with HLM, the major metabolite detected was **9**. A small amount of starting material **3** remained after 60 min, but no diepoxide **13** was detected.

The nonreactivity of **2** to this oxidative system can be explained via a scheme of calculations (Scheme 2). These, analogous to previous work on the similar functional group in 2,3-epoxycinnamic alcohol,⁴³ indicate that oxidation of the alcohol group is hindered by the presence of an adjacent epoxide group. A possible explanation is that the epoxide oxygen can hold back the alcohol proton in a hydrogen-bonded ring system, leading to effective prevention of the oxidation thereof. This stabilization renders **2** unable to form the potent sensitizing metabolite **8** by this mechanism, rendering **2** nonreactive and resulting in unexpectedly low sensitizing potency. The potential side product **15** (Scheme 2), while predicted to be easier to form than **8**, was not searched for in the microsomal incubations, as **2** was not observed to be depleted in this investigation.

KeratinoSens Assay. The full results of the KeratinoSens assay are given in Table 3. Nine of the 10 chemicals classified as sensitizers according to the LLNA were correctly predicted as

Table 2. Peptide Depletion and LLNA EC₃ of Compounds 1–12

Test Compound	Depletion		EC ₃ ^b		Test Compound	Depletion		EC ₃ ^b	
	(%) ^a	(M)	(%)			(%) ^a	(M)	(%)	
1 	0	1.5	22 ^c		7 	79	0.45	7 ^c	
2 	0	3.3	57 ^c		8 	100	0.12	2 ^g	
3 	0	0.42	7 ^d		9 	81	0.082	1.5 ^d	
4 	0	0.93	15 ^e		10 	72	0.29	4.5 ^g	
5 	0	>5.3	NS ^f		11 	0	4.0	60 ^g	
6 	0	>4.7	NS ^f		12 	0	3.4	57 ^g	

^aDepletion after 40 min incubation with AcPHCKRM as described in the text. ^bEC₃ derived from LLNA studies carried out according to standard protocols, as described in the text. ^cRef 5. ^dRef 6. ^ePresent study; full details of results are given in the Supporting Information, Table S1. ^fRef 22. ^gRef 13.

sensitizers by KeratinoSens, with 3 being the only false negative. The two nonsensitizers, 5 and 6, were correctly predicted as nonsensitizers.

The two allylic alcohols, 1 and 4, were predicted to be sensitizing, with a flat dose–response curve. Derivative 2 was also predicted to be sensitizing, though with gene induction occurring only at higher concentration, indicating a weaker sensitization potential as compared to that of 1. This is also in

agreement with the LLNA result and with the low potential for metabolic activation by HLMs of 2. However, overall, the fold-gene induction by these alcohols is low, and this is in contrast to the induction observed by the aldehydes.

Alcohols 5 and 6, which cannot be activated to 2,3-unsaturated aldehydes, do not induce the luciferase gene above the 1.5-fold threshold and thus are predicted to be nonsensitizing. The 6,7-epoxide in 6 does not increase the

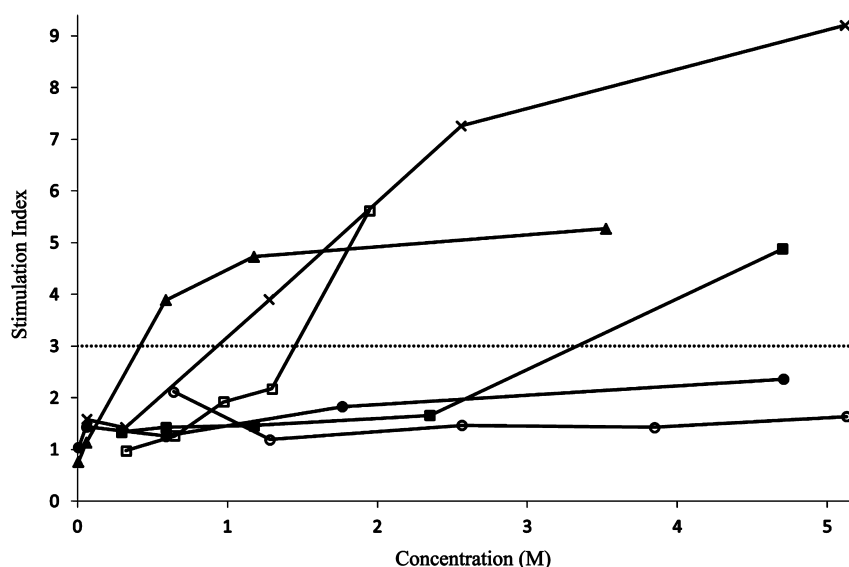


Figure 3. Dose–response curve for 4 (x), as compared to 1 (□), 2 (■), 3 (▲), 5 (○), and 6 (●), all tested in the LLNA. The horizontal dotted line marks a SI of 3, the cutoff concentration limit for a compound to be considered a sensitizer. EC₃ values are as follows: 1, 1.5 M (22% w/v);⁵ 2, 3.3 M (57% w/v);⁵ 3, 0.42 M (7.1% w/v);⁶ 4, 0.93 M (15% w/v); 5, >5.3 M (>80% w/v);²² 6, > 4.7 M (>80% w/v).²² Full LLNA results for 4 can be found in the Supporting Information (Figure S1).

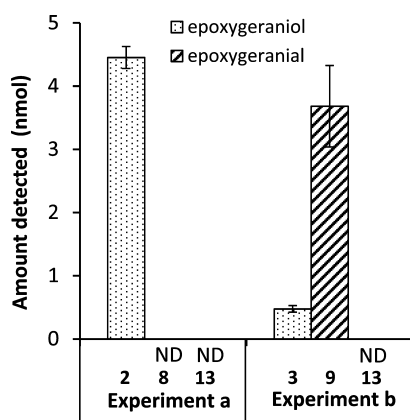


Figure 4. Total amounts of metabolites detected, and remaining starting material, in the incubations of 2 (2,3-epoxyalcohol) (a) and 3 (6,7-epoxyalcohol) (b) with human liver microsomes. ND indicates that the metabolite was not detected. 8 was not detected in the microsomal incubation of 2, and 13 was not detected in either study.

luciferase gene-activation as compared to that of 5. A similar observation is made for the pairs 7 and 9 as well as 11 and 12, which also differ only by the presence of this epoxide. Thus, while the KeratinoSens test system is very sensitive to reactive, skin sensitizing epoxides,^{47,54} the 6,7-epoxide in the current set of chemicals does not activate the Nrf2-response, in line with the LLNA results.

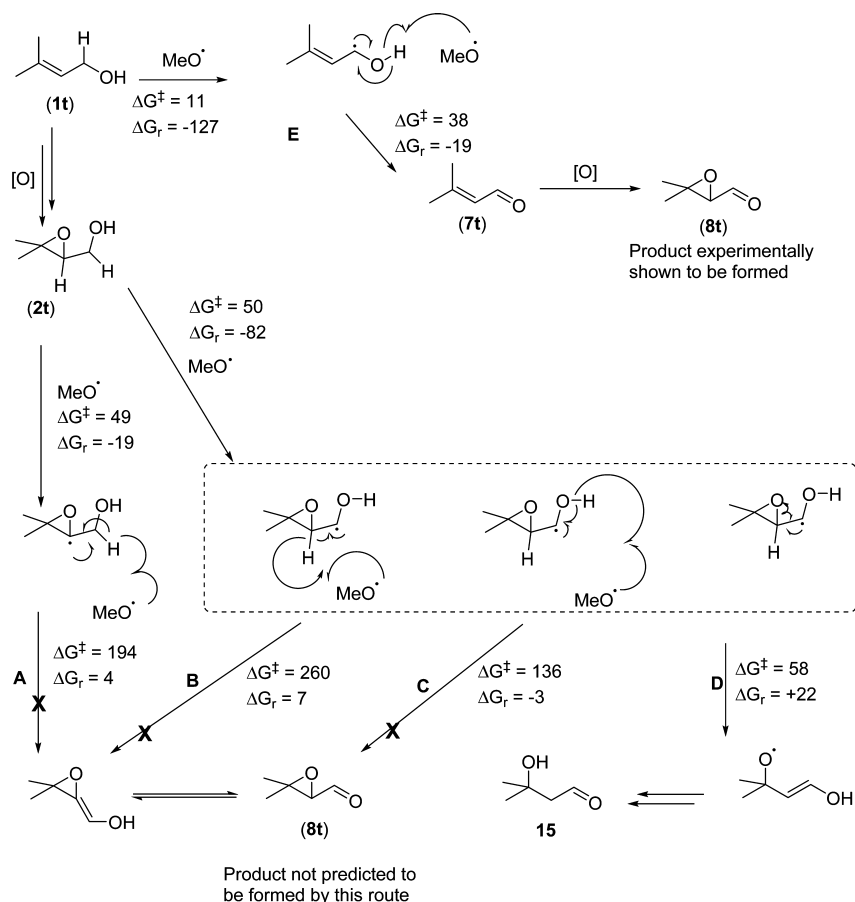
Compounds 7–10, with a 2,3- double bond or a 2,3-epoxide, are all predicted to be strong sensitizers, with 7, 9, and 10 displaying almost congruent dose–response curves (see Table 3 and Figure S2 in the Supporting Information). This indicates that the overall sensitizing potency is similar for these three chemicals, suggesting (in accordance with the other assays and test methods used) that the 2,3-unsaturated aldehyde group is the principal determinant of sensitizing potency. Compound 8 has a similar EC_{KS}1.5 value to that of 7, 9, and 10, but the dose response is steeper (lower EC_{KS}3 and EC_{KS}4.5 values) and cytotoxicity is increased, indicating a potential increase in

sensitizing potency. Compounds 11 and 12, saturated at the 2,3-position, are rated clearly weaker in comparison to 7–10. This is in accordance with the higher LLNA EC₃ and lack of peptide reactivity of 11 and 12 discussed above.

In order to investigate the negative response to 3, the potential for metabolic activation to the potent sensitizer 9 (which had been observed to occur with HLMs; Figure 4) was investigated by retesting compounds 1 and 3 in a recently presented KeratinoSens modification including rat liver S9 fractions.⁴⁶ Indeed, while S9 did not further activate or inactivate 1, an enhanced and clearly positive response was found for 3 (Figure 5). This compound may not be sufficiently activated by the intrinsic metabolic capacity of the KeratinoSens cells, but the additional enzymatic activity in S9 appears to activate it similarly as that shown for the HLM. Thus, the inclusion of the oxidative S9 assay brings the KeratinoSens result for 3 in line with the *in vivo* data.

Structure–Activity Relationships. To summarize the structure–activity findings obtained with the different test methods, the following can be stated. The LLNA indicates that allylic alcohol 1 is a weak sensitizer; however, due to its low intrinsic reactivity, its sensitizing potency must come from oxidized derivatives. 2,3-Epoxy alcohol 2 is prevented from oxidizing into 8 by coordination of the epoxide moiety to the alcohol (resulting in the high $\Delta G^\ddagger$ values shown in Scheme 2). The 2,3-epoxidation thus weakens the molecule as a sensitizer (LLNA data), reinforced by the KeratinoSens assay that showed a much weaker gene induction. This means that, despite being an epoxide (a group conventionally thought to be reactive and sensitizing), oxidation to form 2 results in a decrease in the sensitizing potency of 1. 6,7-Epoxy species 3 can be enzymatically oxidized into 9, and the freely oxidizable 3 is thus a somewhat more potent sensitizer than 1. This is in contrast to the nonobserved oxidation of 2 to 8, since the coordination that allows that is not available. Compound 4 can be oxidized to the potent sensitizer 10, analogous to that for 3 and 1. Alcohol 5 and epoxide 6 lack the unsaturation needed to form the 2,3-unsaturated (or 2,3-epoxy) aldehyde group and

Scheme 2. $\Delta G^\ddagger$ and ΔG_r (all in kJ mol^{-1}) Derived in Jaguar at the B3LYP-D3/6-31+G** Level for Interconversion between Truncated 1, 1t, and the Various Derivatives^a



^aShowing the absence of the conversion of 2 to 8. Structures 1t, 2t, ... indicate truncation of the terpene structure at C4 to speed calculations. All values of $\Delta G^\ddagger$ and ΔG_r are in kJ mol^{-1} .

Table 3. Results from the KeratinoSens Assay

	luciferase induction						cytotoxicity
	I_{max}^a	positive repetitions ^b	classification	$\text{EC}_{\text{KS}1.5} (\mu\text{M})$	$\text{EC}_{\text{KS}3} (\mu\text{M})$	$\text{EC}_{\text{KS}4.5} (\mu\text{M})$	$\text{IC}_{50} (\mu\text{M})$
1	2.2	6/6	sensitizer	24	n.i. ^d	n.i.	1038
2	3.5	6/6	sensitizer	536	1749	n.i.	1602
3	4.6 ^c	2/6	nonsensitizer	928	n.i.	n.i.	>2000
4	1.54	4/6	sensitizer	105	n.i.	n.i.	343
5	1.48	1/5	nonsensitizer	n.i.	n.i.	n.i.	382
6	1.16	0/5	nonsensitizer	n.i.	n.i.	n.i.	>2000
7	57	6/6	sensitizer	10	45	59	148
8	73	6/6	sensitizer	10	19	23	60
9	515	6/6	sensitizer	15	50	65	293
10	4.5	5/5	sensitizer	9.3	42	56	103
11	8.0	5/5	sensitizer	78	175	231	313
12	47	5/5	sensitizer	65	210	320	1151

^a I_{max} is defined as the average maximal induction of gene activity in the test range of 1–2000 μM . ^bIn the prediction model, luciferase induction greater than 1.5-fold at noncytotoxic concentrations and below 1000 μM is considered to be significant for skin sensitization. All assays were performed in triplicate on at least 5 separate occasions. Number of positive repetitions/number of tests is given. ^cFold-induction of >1.5 was noted below 1000 μM in only two repetitions. In two repetitions, $\text{EC}_{\text{KS}1.5}$ was above 1000; therefore, this compound is classified as a nonsensitizer according to the prediction model. ^dn.i. indicates that induction did not achieve the indicated level.

thus they are nonsensitizing and their oxidized derivatives **11** and **12** are only weakly so. Aldehyde **7** and analogues **8–10** are all potent sensitizers. This is reflected in a large peptide depletion, a low EC_3 in the LLNA, and a strong induction in

the KeratinoSens assay. In **8**, sensitization comes from opening of the epoxide at the 2-position (Scheme 1), whereas in **7**, **9**, and **10**, it presumably derives from classic conjugate addition at the 3-position. The similarities in sensitizing potency and

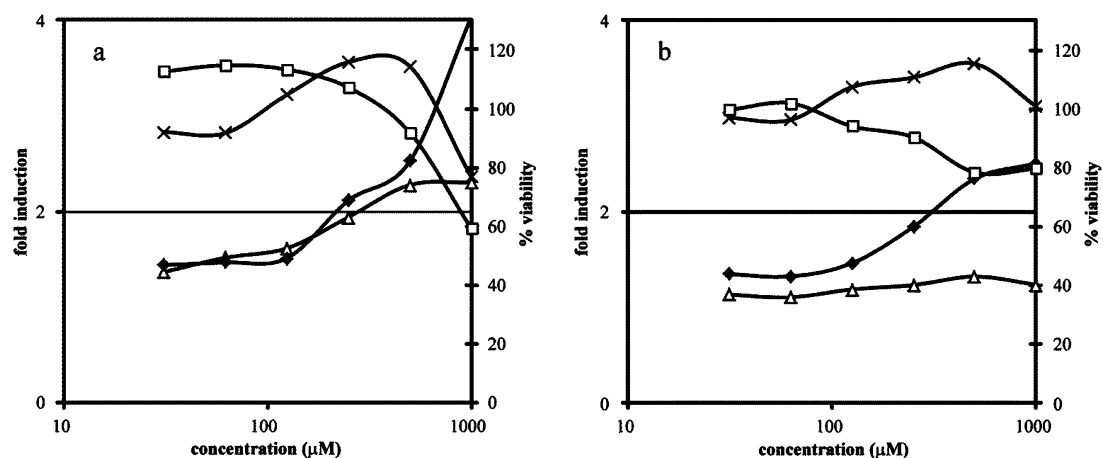


Figure 5.1 (geraniol) (a) and 3 (6,7-epoxyalcohol) (b) tested in KeratinoSens with and without rat liver S9. Shown is luciferase induction with (◆) and without (△) S9 as well as cellular viability with (□) and without (×) S9.

depletion between 1, 3, and 4 as well as 7, 9, and 10 indicate that modification of a remote part of these molecules has a relatively small effect on the overall hazard caused by them.

On the basis of the present investigation, the possible sensitizing derivatives of a 2,3-unsaturated alcohol are, therefore, in order of increasing hazard: (i) the 2,3-epoxy alcohol (might be sensitizing),⁴³ (ii) the 2,3-conjugated aldehyde (well-known skin sensitizer), and (iii) the 2,3-epoxy aldehyde, opened at the 2-position (an even more potent sensitizer by all measures). It is important to note that the formation of a 2,3-epoxy aldehyde by metabolic oxidation must occur via oxidation to the aldehyde first, rather than via the epoxy-alcohol.

To get a complete picture, it is necessary to consider different mechanisms of activation as well as the possibilities for cross reactivity. Epoxidation is described as an important activation route that can occur either metabolically in the skin or by autoxidation outside the skin. A double bond is the activation alert moiety in both cases, but the mechanism is quite different. The metabolic oxidation occurs via an enzymatically catalyzed oxidation of the double bond.^{11,12} Metabolic activation in this study was performed with HLMs (and rat S9 in case of the additional KeratinoSens test) and modeled with methoxyl radicals (Scheme 2); these are easily available surrogate systems, but it should be kept in mind that they must not necessarily reflect the metabolism in the skin. Currently, no analytical data on metabolic conversion of these compounds in the skin or by skin enzymes is available. In autoxidation, the epoxide is secondary to the formation of the primary oxidation products, the hydroperoxides, which are formed on those carbons that lead to a stabilized radical intermediate, in a radical mechanism with hydrogen atom abstraction.³

While patients are conventionally described as allergic to a given allylic alcohol, it is far more likely that they are sensitized to one (or more) of the more potent oxidative derivatives. Indeed, two different patients allergic to the same alcohol could be sensitized to different (though possibly cross-reactive) derivatives of it. However, formation and interconversion of these products from geraniol-containing mixtures, both by autoxidation and by metabolism, would indicate that avoidance of geraniol is still indicated for patients sensitized to any of these metabolites. As seen here, the presence of a 2,3-double bond is critical to the sensitizing potency of the metabolites of an alcohol and thus, *in vivo*, of the alcohol itself.

CONCLUSIONS

Overall, the sensitization potential of the investigated epoxyalcohols and unsaturated alcohols was found to derive from metabolic oxidation to the more potent aldehyde where possible. Where this is less likely, the compound remains weakly or nonsensitizing.

The present investigation confirms the results from our previous study of the corresponding aldehydes:¹³ that non-conjugated nonterminal epoxide moieties have low reactivity and low sensitizing potency. Thus, metabolic activation of a double bond in these positions is not enough to turn a nonsensitizing alcohol into a sensitizer. Additionally, even the 2,3-epoxide moiety formed from an allylic alcohol is not necessarily a potent sensitizer, as shown for 2, where formation of the epoxide weakens the sensitization potential.

In predictive *in vitro* testing, it is important to have a deep understanding of the chemical properties of the compounds studied, e.g., their stability, reactivity, and the possible routes for formation of immunogens. A close interface among chemistry, toxicology, and immunology is therefore important to obtain reliable results *in vivo*, *in vitro*, *in chemico*, and *in silico* for prevention of skin sensitization in the population. Particular care is needed even in the case of series of similar chemicals, such as those described, since apparently small structural changes can alter the reaction chemistry and therefore have dramatic effects on the sensitizing potential.

ASSOCIATED CONTENT

Supporting Information

Complete LLNA information, structure and fragments of the peptide AcPHCKRM, structures of all calculated transition states and intermediates, suggested structures of adducts formed during reactivity experiments, and dose–response curves obtained in the KeratinoSens assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Funding

Financial support for this project was obtained from FORTE: the Swedish Council for Working Life and Social Research and from AFA Försäkring. The work was performed within the Centre for Skin Research (SkinResQU) at the University of Gothenburg, Sweden. KeratinoSens studies were conducted at Givaudan Schweiz AG, Duebendorf, Switzerland.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Suzanne Exing and Anders Eliasson are acknowledged for assistance with the LLNA experiments.

ABBREVIATIONS

ACD, allergic contact dermatitis; AcPHCKRM, Ac-Pro-His-Cys-Lys-Arg-Met-OH; DFT, density functional theory; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; EC₃, estimated concentration required to produce a stimulation index of 3; ESI, electrospray ionization; FCS, fetal calf serum; HEPA, high-efficiency particulate air; HLM, human liver microsomes; LLNA, local lymph node assay; NADPH, nicotinamide adenine dinucleotide phosphate; PBS, phosphate-buffered saline; (Q)SAR, (quantitative) structure–activity relationship; SI, stimulation index

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Supporting Information for ‘Epoxyalcohols: Bioactivation and Conjugation Required for Skin Sensitization’



Supporting Information

- Table S1: Detailed results from the LLNA of 4
- Figure S1: Fragment assignments for Ac-Pro-His-Cys-Lys-Arg-Met (AcPHCKRM)
- Figure S2: KeratinoSensTM dose-response curves
- Associated File: Structures.xyz – contains structures of all transition states, reactants, products and intermediates from computational investigations.

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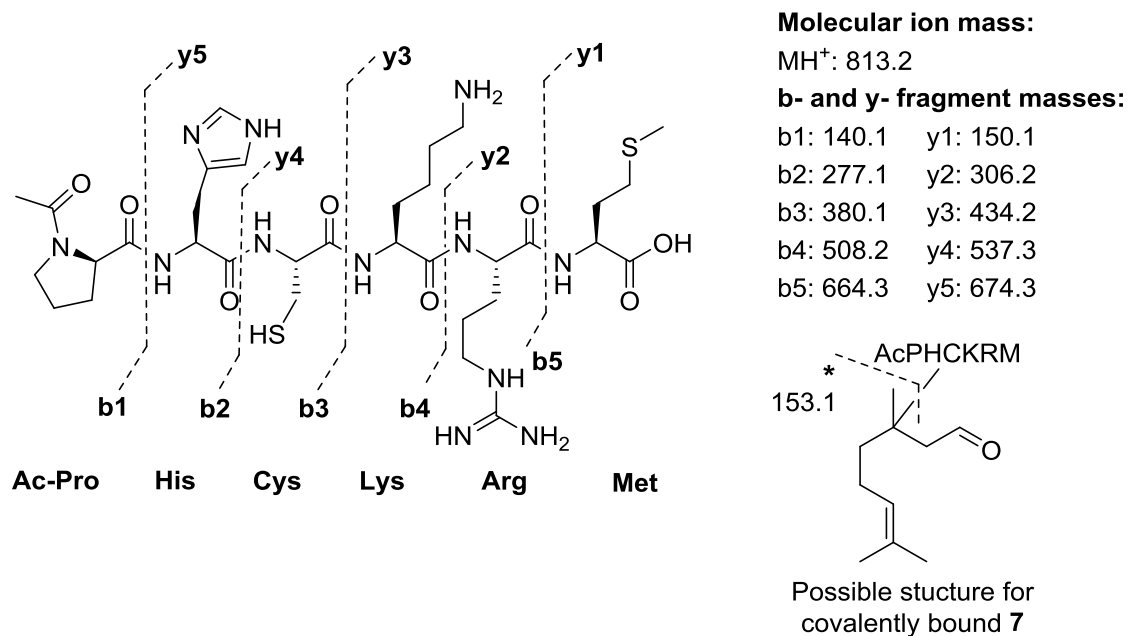
Supporting Information

Table S1. Detailed results from the LLNA of 4^a

Test concentration (% w/v)	Test concentration (M)	[³ H]thymidine incorporation (dpm/lymph node)	SI	EC ₃ value	
				(% w/v)	(M)
Control		665			
1	64*10 ⁻³	1050	1.6		
5	0.32	945	1.4	15	0.93
20	1.3	2592	3.9		
40	2.6	4829	7.3		
80	5.1	6122	9.2		

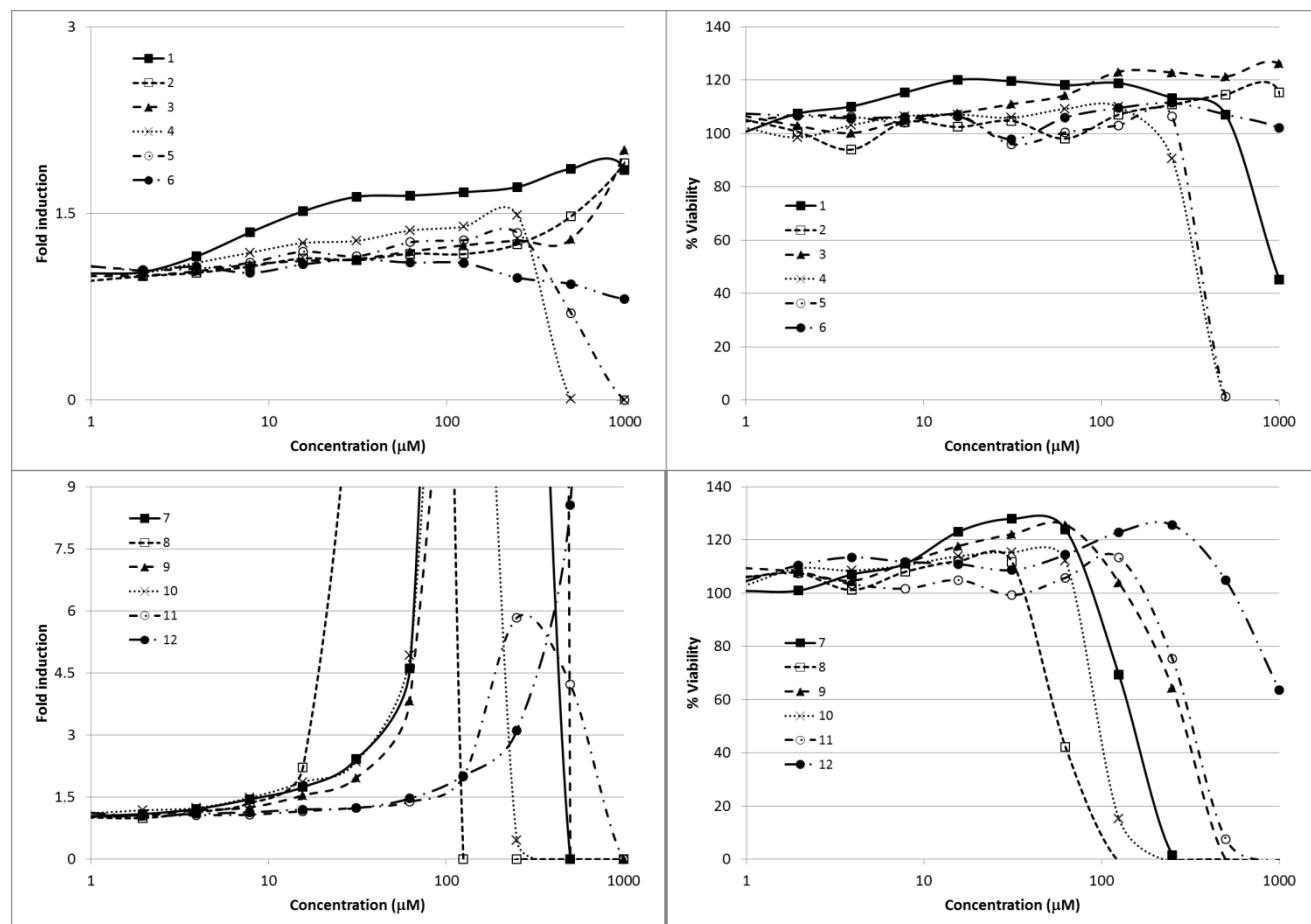
^aGroups of mice were treated with the test substance in five different concentrations, on the dorsum of both ears for three consecutive days. Sham treated control animals received the vehicle alone. On day five, all mice were injected intravenously with PBS (250 μ L) containing 20 μ Ci of [methyl-³H]thymidine. After 5 h the mice were sacrificed, the draining lymph nodes were excised and pooled for each group, single cell suspensions of lymph-node cells were prepared, and the thymidine incorporation into DNA was measured by β -scintillation counting. The increase in thymidine incorporation relative to vehicle-treated controls was derived for each experimental group and recorded as stimulation index (SI). The EC₃ value (the estimated concentration required to induce an SI of 3) was calculated using linear interpolation.

Figure S1. Fragment assignments for Ac-Pro-His-Cys-Lys-Arg-Met (AcPHCKRM)



The haptenated positions on the peptide were determined by analysis of the fragmentation pattern of the mass spectra of conjugates formed between compound and peptide. Peptides are known to form specific fragments by collision-induced decomposition (CID).⁵⁶ The most common fragments observed in the mass spectra of AcPHCKRM conjugates were b- and y-fragments.⁵⁷

Figure S2. Dose-response curves in KeratinoSens™



Dose-response curves in KeratinoSens™ for the six alcohols 1-6 (top) and the six aldehydes 7-12 (bottom). Shown are fold gene induction over solvent control (left) and % viability (right). Note the different scale for gene induction and the fact that for the aldehydes gene induction is off-scale (See also Table 2 for I_{max}).

Given are the average values at each concentration for all repetitions done.

22

DJP-018- prod	0.0000000		
C	1.24490	2.51540	2.71420
O	1.63420	3.42960	3.75280
H	1.87640	2.66910	1.82860
C	1.35530	1.06610	3.21420
C	2.79960	0.67440	3.71400
C	2.73700	-0.70710	4.39470
H	3.72740	-0.96650	4.78470
H	2.03350	-0.68500	5.23510
H	2.42530	-1.49620	3.70190
C	3.79390	0.60580	2.53700
H	4.78960	0.36050	2.92200
H	3.86010	1.56660	2.01570
H	3.51470	-0.16740	1.81200
O	3.21810	1.65380	4.65870
H	0.21040	2.73750	2.42520
H	2.35730	2.89410	4.24290
C	0.98080	0.53100	0.31610
S	0.56620	-0.10220	1.99160
H	0.52880	-0.17940	-0.38400
H	2.05860	0.55900	0.14380
H	0.68980	0.97070	4.08330
H	0.54840	1.51780	0.13720

22

DJP-019- prod	0.0000000		
C	1.14140	2.51620	2.84610
O	0.63190	3.56090	3.70370
H	2.03030	2.88650	2.31610
C	1.48630	1.29260	3.72370
C	2.19520	0.14080	2.91950
C	2.27120	-1.09960	3.82640
H	2.78990	-0.83340	4.75200
H	1.27130	-1.46120	4.09820
H	2.81250	-1.91680	3.33950
C	3.59280	0.52860	2.43060
H	4.19560	0.82090	3.29580
H	3.57640	1.37360	1.73680
H	4.08360	-0.31460	1.93190
H	0.50420	0.86330	4.02370
O	2.21490	1.70860	4.84420
H	0.35940	2.28710	2.11580
H	1.13940	3.42960	4.53430
C	1.59710	0.53640	-0.00610
S	1.12650	-0.44610	1.47120
H	2.65160	0.40190	-0.25650
H	1.37440	1.59890	0.11160
H	0.99350	0.13960	-0.82950

22

DJP-041DJP-018-RXN_+_0_5.01_5543.01	0.0000000		
C	1.70470	3.03960	2.82790
O	1.55630	4.45550	3.07620
H	2.52840	2.88420	2.12610
C	1.90700	2.29850	4.12730
C	3.05090	1.40830	4.43660
C	2.88110	0.34830	5.50690
H	3.80740	0.25760	6.08880
H	2.06610	0.60420	6.19240
H	2.66410	-0.62050	5.04380
C	4.17080	1.12800	3.45770

H	5.11870	1.06510	4.00760
H	4.28130	1.90800	2.69910
H	3.98840	0.16300	2.97200
O	2.99660	2.77390	4.95000
H	0.77540	2.70290	2.35940
H	2.38190	4.78640	3.47700
C	1.52790	-0.16010	0.86720
S	2.01100	-1.86040	1.45090
H	1.53460	-0.09940	-0.22610
H	2.21550	0.59980	1.24620
H	0.99120	2.12790	4.70060
H	0.52190	0.10020	1.20870

22

DJP-041DJP-019-RXN+_0_5.01_5546.01 0.0000000

C	2.19830	2.98890	4.19360
O	2.00720	3.33930	5.58160
H	3.26560	2.98460	3.95990
C	1.54330	1.66090	3.89320
C	2.15350	0.56160	3.11470
C	1.23710	-0.42820	2.40960
H	0.59050	-0.92660	3.13840
H	0.60160	0.03670	1.65190
H	1.82300	-1.20780	1.91400
C	3.53320	0.68870	2.50120
H	4.01630	-0.29490	2.47670
H	4.17920	1.37480	3.05150
H	3.44230	1.03790	1.46580
H	0.45110	1.68460	3.95240
O	2.13000	0.51530	4.57750
H	1.71490	3.77370	3.60270
H	2.58350	2.77230	6.12570
C	-0.99140	-0.93350	-2.61490
S	-0.24700	-0.57550	-0.94460
H	-1.35380	-1.96420	-2.68280
H	-0.25700	-0.79540	-3.41460
H	-1.83840	-0.27380	-2.82430

22

DJP-018-RXN+_0_5.01 - TS Guess 0.0000000

C	1.95420	2.50420	2.46080
O	2.22610	3.88240	2.80860
H	2.68240	2.17990	1.70980
C	1.98840	1.58020	3.66730
C	3.22200	1.23350	4.39140
C	3.13480	0.05380	5.35040
H	3.94190	0.12090	6.08840
H	2.17600	0.05190	5.87860
H	3.24180	-0.88960	4.80480
C	4.52960	1.24900	3.61060
H	5.37420	1.22850	4.30770
H	4.61590	2.14590	2.99140
H	4.58690	0.36720	2.96410
O	2.88810	2.49060	5.00050
H	0.95720	2.46290	2.01450
H	2.71000	3.84180	3.66190
C	1.52850	-0.16610	0.39590
S	1.46540	-0.59690	2.19650
H	1.84030	-1.03140	-0.19780
H	2.25220	0.63640	0.22500
H	1.05270	1.36170	4.16630
H	0.55620	0.16970	0.02290

22

DJP-019-RXN_+_0_5.01 - TS	Guess	0.0000000
C	1.15500	2.43690
O	1.17250	3.42310
H	1.94390	2.66260
C	1.32800	1.04990
C	1.90310	-0.06780
C	1.63010	-1.47090
H	2.41630	-1.77470
H	0.66240	-1.55090
H	1.65760	-2.15990
C	3.02410	0.11260
H	3.96480	-0.20930
H	3.13620	1.14240
H	2.84850	-0.53520
H	0.39470	0.76840
O	2.47670	0.92470
H	0.18220	2.51480
H	2.00390	3.30700
C	0.46230	0.92420
S	-0.15130	-0.23380
H	1.29880	0.48610
H	0.80730	1.86250
H	-0.32590	1.16050

20

DJP-020- prod	0.0000000
C	-1.75980
O	-2.61320
H	-2.00880
C	-0.33060
C	0.24040
C	1.66410
H	1.63550
H	2.07230
H	2.34820
C	0.34740
H	1.00930
H	0.76340
H	-0.63150
H	-0.24110
O	-0.62670
C	0.02200
S	0.52860
H	0.47850
H	0.38530
H	-1.06450

20

DJP-021- prod	0.0000000
C	-0.15730
O	-0.94250
H	0.92370
C	-0.55910
C	0.02910
C	-0.50060
H	-0.21010
H	-0.08650
H	-1.59520
C	1.56020
H	1.92960
H	1.94110

H	1.97570	4.50120	1.68590
H	-1.65850	5.53620	2.42900
O	-0.15200	6.81300	3.12100
C	0.37220	2.36430	0.96190
S	-0.68870	3.75190	0.39520
H	0.36440	2.24800	2.04770
H	1.39830	2.46920	0.60260
H	-0.06160	1.46400	0.51300
20			
DJP-041DJP-020-RXN+_0_5.01_5549.01		0.0000000	
C	-1.95110	5.04160	2.62270
O	-2.32200	4.84990	3.77390
H	-2.65510	5.39040	1.84450
C	-0.54730	4.80820	2.19070
C	0.25150	5.88100	1.52200
C	1.75790	5.78680	1.67130
H	2.07980	4.74140	1.67570
H	2.24620	6.30160	0.83490
H	2.07900	6.25990	2.60460
C	-0.30080	7.26490	1.25770
H	-0.07550	7.91600	2.10980
H	0.18040	7.68610	0.36730
H	-1.38180	7.25890	1.09720
H	0.00110	4.05290	2.75550
O	-0.33820	4.80140	0.76100
C	0.89570	1.63390	1.28040
S	1.70060	1.22190	2.90800
H	1.05610	0.83090	0.55400
H	1.30490	2.55750	0.85740
H	-0.18560	1.76340	1.39250
20			
DJP-041DJP-021-RXN+_0_5.01_5552.01		0.0000000	
C	-0.29420	4.12330	3.13950
O	-1.15070	3.39210	3.61480
H	0.78100	3.93630	3.31750
C	-0.63950	5.32060	2.32440
C	0.11350	5.71720	1.09450
C	-0.63260	6.54760	0.07300
H	0.05990	7.24110	-0.42000
H	-1.06090	5.88810	-0.68980
H	-1.43840	7.12070	0.54000
C	1.27490	4.91580	0.54710
H	0.89380	4.16640	-0.15630
H	1.94310	5.58770	-0.00420
H	1.85330	4.43640	1.33940
H	-1.67990	5.63520	2.41510
O	0.34600	6.38640	2.35820
C	-0.07240	1.48390	1.06610
S	-1.22490	1.77040	-0.36130
H	0.59850	2.33500	1.20800
H	0.54730	0.59620	0.90310
H	-0.62350	1.34000	2.00020
20			
DJP-020-RXN+_0_5.01 - TS		Guess	0.0000000
C	-1.16340	3.95480	1.36700
O	-1.55260	2.94910	0.76980
H	-1.74560	4.38220	2.19510
C	-0.01180	4.76580	0.84620
C	0.57260	5.94420	1.52000
C	1.97570	6.35710	1.12620

H	2.21140	6.03620	0.10710
H	2.07760	7.44740	1.18690
H	2.69790	5.90460	1.81420
C	0.13200	6.41500	2.88950
H	0.78450	5.98710	3.65800
H	0.22190	7.50680	2.93990
H	-0.90110	6.14310	3.11470
H	0.58340	4.24530	0.09290
O	-0.41200	6.12430	0.45360
C	-0.08570	1.13500	2.92760
S	0.43250	2.77550	3.61950
H	0.50300	0.87720	2.04240
H	-1.13560	1.17140	2.62320
H	0.03110	0.33040	3.65970
20			
DJP-021-RXN_+_0_5.01 - TS		Guess	0.0000000
C	-0.25810	3.74230	3.04510
O	-1.00470	2.96700	3.62410
H	0.82970	3.72760	3.24430
C	-0.75260	4.83750	2.12300
C	-0.03970	5.10950	0.86470
C	-0.74590	5.91570	-0.18800
H	-0.33660	6.93260	-0.20640
H	-0.55620	5.47750	-1.17330
H	-1.82360	5.96330	-0.01390
C	1.44050	4.91280	0.69290
H	1.62900	4.57810	-0.33080
H	1.94760	5.87600	0.82570
H	1.87660	4.18830	1.38320
H	-1.84250	4.75670	2.02010
O	-0.26250	6.10150	2.51710
C	-0.11750	1.24800	0.76880
S	-0.78990	2.60700	-0.29910
H	0.83330	1.54930	1.22030
H	0.05890	0.33720	0.18810
H	-0.80820	1.00470	1.58100
21			
67 epoxy 2 pos end		0.0000000	
C	-1.44950	0.03140	1.68850
C	0.04530	-0.47000	1.53420
H	-0.02290	-1.52520	1.24020
O	-2.11300	-0.82180	2.59280
C	-2.15220	-0.00660	0.30790
H	-2.05970	-1.00040	-0.14600
H	-3.21650	0.21700	0.44350
H	-1.74560	0.72520	-0.40140
C	-1.47400	1.49580	2.19140
H	-2.50710	1.86080	2.20800
H	-1.07350	1.56870	3.20680
H	-0.89040	2.16470	1.54630
C	0.86030	-0.34620	2.82270
H	1.11320	0.69780	3.03880
H	0.27540	-0.74140	3.65930
H	1.79390	-0.91370	2.76590
C	2.60370	-0.39510	0.14530
H	3.18650	-0.09160	1.01800
H	3.12270	-0.05580	-0.75710
S	0.94040	0.38460	0.11990
H	2.52410	-1.48560	0.11330

67 epoxy 3 pos end	0.0000000	
C	-0.90870	0.21900 1.33070
C	0.47950	-0.42190 1.73590
H	0.90760	0.29740 2.46620
O	0.31650	-1.68930 2.33270
C	-1.78000	0.39260 2.57970
H	-1.28650	1.02550 3.32630
H	-2.74640	0.84330 2.32750
H	-1.95600	-0.58570 3.03890
C	-1.64650	-0.62130 0.27550
H	-2.65230	-0.23000 0.08930
H	-1.11290	-0.64950 -0.67990
H	-1.73990	-1.64780 0.64040
C	1.47260	-0.47880 0.55800
H	1.10990	-1.14470 -0.23340
H	1.64960	0.50770 0.11570
H	2.43110	-0.86880 0.91490
C	0.06080	3.03500 1.68810
H	0.04180	4.02650 1.22410
H	-0.49270	3.08600 2.62900
S	-0.73890	1.90380 0.48590
H	1.10020	2.75710 1.88220

21		
67 epoxy 2 pos start	0.0000000	
C	-1.92490	0.44730 1.67050
C	-0.70710	-0.15370 2.25030
H	-0.47340	-1.16770 1.91700
O	-1.94510	-0.10280 3.03320
C	-2.73680	-0.36020 0.67780
H	-2.57310	-1.43320 0.81660
H	-3.80740	-0.14850 0.79470
H	-2.44740	-0.09490 -0.34590
C	-2.12730	1.94450 1.58680
H	-3.19330	2.18330 1.68320
H	-1.58500	2.47930 2.36890
H	-1.78530	2.31140 0.61240
C	0.47800	0.59820 2.79900
H	0.22490	1.61720 3.10240
H	0.88180	0.06670 3.66970
H	1.26950	0.64350 2.04210
C	4.14160	0.39770 -2.34110
H	5.03410	1.00910 -2.18070
H	3.62440	0.78910 -3.22230
S	3.03440	0.41770 -0.84190
H	4.47320	-0.61770 -2.57570

21		
67 epoxy 3 pos start	0.0000000	
C	-1.48860	-0.42090 1.28670
C	-0.02170	-0.50780 1.40240
H	0.38760	-0.32270 2.39740
O	-0.82480	-1.73390 1.35190
C	-2.30240	-0.09160 2.52050
H	-1.76210	-0.36110 3.43300
H	-2.51620	0.98300 2.54680
H	-3.25820	-0.62990 2.50230
C	-2.17110	-0.10700 -0.02640
H	-2.30080	0.97730 -0.11840
H	-1.59640	-0.46100 -0.88380
H	-3.16200	-0.57440 -0.05680
C	0.96090	-0.28290 0.28360

H	0.54470	-0.54320	-0.69250
H	1.25360	0.77340	0.26850
H	1.86210	-0.88480	0.44850
C	0.59160	5.56660	0.62710
H	1.61580	5.86070	0.37580
H	-0.07190	6.00490	-0.12490
S	0.41060	3.71120	0.68630
H	0.34500	6.02110	1.59130
21			
67 epoxy 2 pos ts		0.0000000	
C	-1.42050	-0.09170	1.54020
C	-0.18650	-0.60700	2.16410
H	0.15520	-1.57180	1.80840
O	-2.02420	-1.02410	2.43860
C	-1.63060	-0.44160	0.06960
H	-1.27800	-1.45590	-0.14530
H	-2.70020	-0.38200	-0.16500
H	-1.09660	0.25960	-0.58000
C	-1.77870	1.36560	1.82520
H	-2.80200	1.56760	1.48980
H	-1.71480	1.58960	2.89320
H	-1.09710	2.03280	1.28530
C	0.38680	-0.06520	3.44370
H	0.59970	1.00630	3.36730
H	-0.33130	-0.19930	4.25920
H	1.30720	-0.59120	3.70590
C	1.78680	-0.32040	-1.01940
H	1.54860	-1.38740	-0.98420
H	2.74690	-0.20850	-1.53310
S	1.86620	0.39540	0.68910
H	1.02150	0.17540	-1.62380
21			
67 epoxy 3 pos ts		0.0000000	
C	-1.10360	0.56720	1.17220
C	0.25630	0.14360	1.57400
H	0.67190	0.80710	2.34840
O	-0.38140	-1.00220	2.11580
C	-1.98830	1.22390	2.19600
H	-1.40610	1.66680	3.00900
H	-2.59160	1.99940	1.71800
H	-2.67510	0.48070	2.61760
C	-1.76640	0.05010	-0.07410
H	-2.49700	0.78130	-0.43080
H	-1.05390	-0.15970	-0.87390
H	-2.31500	-0.86870	0.16200
C	1.31920	-0.13550	0.52600
H	0.94840	-0.81640	-0.24560
H	1.63610	0.79710	0.04960
H	2.18540	-0.60070	1.00900
C	0.60180	3.66300	1.40820
H	0.86400	4.71930	1.28770
H	0.02400	3.56080	2.33220
S	-0.37500	3.03740	-0.03280
H	1.53040	3.09520	1.52590
22			
001 in		0.0000000	
C	-2.46360	-3.24100	2.81870
H	-1.79800	-3.93020	3.38500
H	-2.85480	-2.51260	3.54590
H	-3.26780	-3.87720	2.41160

O	-1.67410	-2.68250	1.84360
C	0.35960	2.27740	0.40520
O	-0.40250	2.85970	-0.66150
H	1.37620	2.68520	0.41880
C	1.56720	-0.08980	0.40690
C	2.95050	0.46190	0.68980
H	3.08060	1.47810	0.31340
H	3.14300	0.45750	1.76870
H	3.70450	-0.17520	0.21250
C	1.38450	-1.54410	0.78030
H	2.08280	-2.16490	0.20720
H	1.58930	-1.69980	1.84600
H	0.36560	-1.87780	0.57380
O	1.01820	0.25230	-0.90170
H	-0.13090	2.56640	1.34140
H	0.08690	2.69480	-1.48650
C	0.36860	0.76940	0.29190
H	-0.59730	0.29730	0.50360
22			
001 out	0.0000000		
C	-0.99070	-2.21860	2.49050
H	-0.75140	-3.08720	1.86240
H	-0.05100	-1.71670	2.75730
H	-1.45940	-2.58110	3.41020
O	-1.91530	-1.32850	1.85680
C	-0.06080	1.58020	1.18800
O	-0.53460	2.64800	0.37230
H	0.84730	1.86800	1.74610
C	1.42030	-0.46870	0.13930
C	2.78440	-0.01010	0.59940
H	2.82500	1.07620	0.71770
H	3.02460	-0.47670	1.56160
H	3.54940	-0.31380	-0.12490
C	1.28600	-1.93330	-0.20620
H	1.99050	-2.19910	-1.00220
H	1.52490	-2.54260	0.67200
H	0.27090	-2.17150	-0.53450
O	0.79680	0.45300	-0.87830
H	-0.83690	1.36000	1.92870
H	0.17250	2.88320	-0.25430
C	0.22600	0.34240	0.37330
H	-1.47600	-0.95620	1.06990
22			
001 ts	0.0000000		
C	-1.91210	-1.91360	1.51210
H	-2.71920	-1.23760	1.19820
H	-1.44230	-2.35420	0.62090
H	-2.35680	-2.73960	2.08640
O	-0.96590	-1.28020	2.35290
C	0.12570	1.71930	1.15120
O	-1.01610	2.22120	0.45210
H	1.01960	2.31210	0.91500
C	1.56670	-0.36790	0.20240
C	2.70120	0.47470	-0.33260
H	2.36880	1.47360	-0.62810
H	3.47900	0.57540	0.43390
H	3.14660	-0.01420	-1.20740
C	1.88350	-1.82230	0.45670
H	2.21780	-2.29820	-0.47330
H	2.69500	-1.89790	1.18910

H	1.01680	-2.36180	0.84450
O	0.27020	-0.14740	-0.51040
H	-0.07370	1.82560	2.22100
H	-0.80650	2.28980	-0.49670
C	0.38150	0.25370	0.82720
H	-0.25720	-0.48560	1.61380
21			
002 in	0.0000000		
C	0.19650	3.99990	-0.33010
H	0.99160	3.53500	0.26580
H	0.64630	4.55980	-1.15770
H	-0.35520	4.69480	0.30820
O	-0.74280	3.02060	-0.80970
C	0.18620	1.07290	1.66250
O	-1.01830	1.73590	1.61730
H	-0.23480	2.35630	-1.30980
C	0.88330	0.64090	0.62010
C	2.00240	0.04450	-0.05780
C	3.27810	0.82780	-0.26050
H	3.09340	1.90370	-0.21190
H	3.99350	0.56110	0.52610
H	3.73060	0.58080	-1.22760
C	2.10250	-1.45660	-0.19650
H	2.54260	-1.72490	-1.16370
H	2.75400	-1.84870	0.59300
H	1.12040	-1.92640	-0.10270
O	0.79210	0.64120	-0.76580
H	0.55670	0.89600	2.66800
H	-1.14730	2.11100	0.71260
21			
002 out	0.0000000		
C	0.40140	3.96020	-0.16040
H	1.04590	3.45700	0.56970
H	1.02340	4.53250	-0.85750
H	-0.25720	4.65030	0.37330
O	-0.43660	3.02010	-0.85710
C	0.05430	0.93770	1.62410
O	-1.13450	1.59890	1.41280
H	0.14950	2.36580	-1.28080
C	0.91080	0.55030	0.68890
C	2.13880	0.00670	0.17350
C	3.40830	0.82510	0.20630
H	3.18770	1.89310	0.27550
H	4.00530	0.53470	1.07820
H	4.00690	0.63660	-0.69220
C	2.29980	-1.48410	-0.01100
H	2.88370	-1.69810	-0.91340
H	2.84010	-1.89750	0.84850
H	1.32810	-1.97880	-0.08220
O	1.04170	0.61240	-0.69210
H	0.26450	0.72560	2.66750
H	-1.11290	2.02100	0.52070
21			
002 ts	0.0000000		
C	-0.32730	4.73710	0.35540
H	-0.59040	4.38460	1.37500
H	0.48200	5.48040	0.49690
H	-1.21050	5.31460	0.01300
O	0.02480	3.70380	-0.51850
C	0.15960	1.51480	0.92380

O	-1.13890	1.72480	0.44380
H	0.71790	2.45080	0.50770
C	0.86830	0.45510	0.26750
C	2.10620	-0.26890	0.12140
C	3.27060	0.41080	-0.52700
H	2.98320	1.34400	-1.01940
H	3.98890	0.64390	0.27170
H	3.75870	-0.26100	-1.24130
C	2.24860	-1.64620	0.68310
H	2.69890	-2.30930	-0.06320
H	2.93350	-1.58060	1.53960
H	1.29410	-2.05270	1.02490
O	0.68080	-0.13950	-0.83650
H	0.29190	1.49060	2.01370
H	-0.92700	2.58480	-0.11680
22			
003 in	0.0000000		
C	1.93360	5.37320	-2.42770
H	1.40560	6.09210	-3.09350
H	2.15510	5.93260	-1.50350
H	2.85780	5.08450	-2.95330
O	1.05650	4.33230	-2.24040
C	0.69210	-0.48050	1.09560
C	1.28350	0.27190	-0.02660
C	0.66030	1.52910	-0.59830
H	-0.41960	1.57640	-0.44860
H	1.10880	2.41410	-0.13510
H	0.85830	1.58870	-1.67420
C	2.77690	0.15390	-0.26250
H	3.00150	0.22060	-1.33360
H	3.30450	0.96890	0.24540
H	3.15870	-0.79860	0.11650
H	1.38110	-1.07720	1.70150
O	0.54780	-0.96820	-0.26530
C	-0.56570	-0.09960	1.84330
H	-1.33830	0.23710	1.14550
H	-0.34020	0.73150	2.52650
O	-1.11860	-1.21820	2.55360
H	-0.52570	-1.42700	3.29640
22			
003 out	0.0000000		
C	-1.08340	3.14640	-1.41410
H	-1.36760	2.98820	-2.46080
H	-1.70000	2.50150	-0.77520
H	-1.27450	4.18950	-1.14850
O	0.32060	2.89830	-1.21520
C	0.43250	-0.00340	0.74030
C	1.70900	-0.21240	-0.00650
C	2.83840	0.79330	0.03210
H	2.50260	1.78650	0.32760
H	3.59840	0.46330	0.74870
H	3.31150	0.85740	-0.95470
C	2.13550	-1.63230	-0.31730
H	2.67520	-1.66470	-1.27160
H	2.80740	-2.00220	0.46530
H	1.27050	-2.29920	-0.38160
H	-0.10680	-0.91620	1.00340
O	0.51260	0.20410	-0.73380
C	0.04250	1.10990	1.59990
H	0.46940	1.93880	-1.36470

H	-0.35810	0.88150	2.58230
O	0.43930	2.40390	1.44610
H	0.49830	2.64780	0.48430
22			
003 ts	0.0000000		
C	0.61270	4.07440	-0.83680
H	1.23980	3.69380	-1.65400
H	-0.44320	3.84990	-1.05360
H	0.69540	5.17370	-0.80420
O	0.99710	3.57730	0.42310
C	0.67530	0.19240	0.89460
C	1.63260	-0.16840	-0.17760
C	1.72450	0.62460	-1.46310
H	0.80760	1.17170	-1.69060
H	2.54700	1.34430	-1.38670
H	1.94100	-0.05100	-2.29880
C	2.86970	-0.96180	0.18140
H	3.11350	-1.65790	-0.63070
H	3.72010	-0.28470	0.31840
H	2.72090	-1.53350	1.10240
H	0.93010	-0.08790	1.91890
O	0.38460	-0.92260	0.01050
C	-0.30450	1.34470	0.79940
H	0.34160	2.34270	0.61040
H	-0.78690	1.56910	1.75830
O	-1.26970	1.21500	-0.20440
H	-1.10740	0.39450	-0.71350
16			
004 in	0.0000000		
C	0.20930	1.30190	1.09090
O	-1.05020	1.51280	1.58110
H	0.77500	2.21720	0.95680
C	0.61420	0.01020	0.58350
C	1.96070	-0.33560	0.07110
C	3.08540	0.68230	0.01490
H	2.71290	1.69130	-0.17680
H	3.64880	0.68590	0.95460
H	3.78110	0.41640	-0.79060
C	2.42620	-1.77340	0.21060
H	3.07770	-2.04440	-0.62890
H	2.99630	-1.90200	1.13750
H	1.57410	-2.45930	0.22650
H	0.00680	-0.82340	0.94460
O	0.91310	-0.14190	-0.90510
H	-1.53910	0.67420	1.68230
16			
finalSP004-out.01_961.01	0.0000000		
C	0.23200	1.08040	1.21180
O	-0.88290	1.23930	1.94040
H	0.85270	1.96410	1.21530
C	0.52090	-0.05200	0.54820
C	1.75860	-0.31990	-0.29570
C	2.75410	0.85230	-0.31870
H	2.29510	1.75750	-0.71790
H	3.11790	1.07380	0.68520
H	3.62520	0.62460	-0.93590
C	2.44730	-1.60800	0.17300
H	3.31760	-1.84750	-0.44050
H	2.78670	-1.51100	1.20480
H	1.76570	-2.45860	0.12770

H	-0.17840	-0.87160	0.61250
O	1.35730	-0.53970	-1.62540
H	-0.92840	2.14550	2.21010
16			
004 ts	0.0000000		
C	0.19930	1.19460	1.00680
O	-1.08070	1.29430	1.45810
H	0.75940	2.11550	0.89010
C	0.67530	-0.08630	0.61740
C	1.99460	-0.34160	-0.00820
C	3.01610	0.78160	-0.11440
H	2.54570	1.73600	-0.36380
H	3.56260	0.88970	0.82920
H	3.73980	0.54310	-0.90280
C	2.63570	-1.70830	0.19150
H	3.31380	-1.91970	-0.64510
H	3.22380	-1.72800	1.11630
H	1.88100	-2.49950	0.23570
H	0.10570	-0.95410	0.94970
O	0.97430	-0.31620	-1.01890
H	-1.30090	2.21430	1.70220
21			
006 in	0.0000000		
C	-1.41340	3.58270	-1.66170
H	-0.75830	2.84960	-2.15320
H	-2.44290	3.20110	-1.70640
H	-1.37020	4.51330	-2.23490
O	-0.99800	3.87350	-0.32530
C	0.36160	0.91900	-0.15130
O	-0.79240	1.05870	0.23090
H	0.98530	1.78970	-0.42730
C	0.99090	-0.42180	-0.27300
C	2.31830	-0.71110	0.35430
C	3.07200	0.34130	1.14290
H	2.89690	1.34870	0.75900
H	2.76590	0.30740	2.19420
H	4.14710	0.13540	1.09580
C	2.61910	-2.14670	0.73010
H	3.69390	-2.33730	0.62530
H	2.34110	-2.32890	1.77360
H	2.07360	-2.84600	0.09000
H	0.28890	-1.24740	-0.40530
O	2.19840	-0.47250	-1.06450
H	-1.09290	3.04060	0.17220
21			
006 out	0.0000000		
C	0.25770	2.29950	-2.62960
H	1.01070	1.51090	-2.50880
H	-0.69000	1.82230	-2.91630
H	0.56950	2.95710	-3.44740
O	0.12290	3.10170	-1.45530
C	0.04260	0.77170	1.62580
O	-0.28670	1.84070	1.13300
H	-0.02600	0.60690	2.71710
C	0.56470	-0.39400	0.86310
C	1.82680	-0.31500	0.05780
C	2.60950	0.97320	-0.06890
H	1.95700	1.84640	-0.06290
H	3.33290	1.05450	0.74910
H	3.16760	0.96700	-1.01170

C	2.62670	-1.58840	-0.11820
H	3.11700	-1.58440	-1.09900
H	3.40520	-1.65110	0.65020
H	1.98660	-2.47240	-0.04790
H	0.30700	-1.36160	1.30130
O	0.52600	-0.31980	-0.57160
H	-0.13890	2.51630	-0.72150
21			
006 ts	0.0000000		
C	-1.02010	2.36460	-1.59150
H	-0.63240	3.27240	-2.07960
H	0.14510	1.96890	-1.06600
H	-1.15620	1.50530	-2.26710
O	-1.84150	2.49990	-0.58410
C	0.69060	1.63410	0.10320
O	-0.22260	1.91230	1.00900
H	1.54660	2.31890	0.02240
C	0.95710	0.17540	-0.15320
C	2.27280	-0.47680	0.06290
C	3.48180	0.28820	0.55670
H	3.46230	1.33540	0.24870
H	3.53000	0.24320	1.65030
H	4.39460	-0.16900	0.15850
C	2.29520	-1.96590	0.33660
H	3.18260	-2.41680	-0.12350
H	2.34200	-2.14820	1.41580
H	1.40370	-2.45610	-0.06530
H	0.05660	-0.43960	-0.07270
O	1.81170	-0.12350	-1.27490
H	-1.18060	2.22550	0.40190
21			
007 in	0.0000000		
C	-0.55860	-2.26930	2.55770
H	-0.96700	-3.27000	2.72580
H	-1.27410	-1.53030	2.93970
H	-0.45250	-2.11200	1.47680
O	0.70090	-2.20450	3.23760
C	0.18860	0.89970	1.63540
O	-1.18310	1.06130	1.66330
H	0.69790	1.37270	2.46930
C	0.86390	0.23470	0.70810
C	2.01000	-0.28370	0.00790
C	2.83130	0.62210	-0.88080
H	2.25850	1.50000	-1.19010
H	3.71710	0.95910	-0.32980
H	3.17280	0.07830	-1.76920
C	2.66190	-1.58170	0.42600
H	2.87900	-2.18910	-0.46030
H	3.61250	-1.36720	0.92710
H	2.03020	-2.14560	1.11450
H	1.03220	-1.29700	3.14100
O	0.56690	-0.45320	-0.45420
H	-1.58360	0.74050	0.83510
21			
007-TS-SPE.01	0.0000000		
C	0.04010	-2.02670	3.00690
H	-0.63680	-2.60340	3.65400
H	1.00340	-2.55120	2.95920
H	0.18610	-1.04220	3.47280
O	-0.58710	-1.93370	1.73810

C	-0.05310	0.96390	1.06390
O	-1.28990	0.76240	1.60040
H	0.23540	2.00280	0.94360
C	0.73610	-0.15550	0.56970
C	2.17550	-0.28760	0.26570
C	3.08020	0.92190	0.24340
H	2.52240	1.84020	0.04260
H	3.58800	1.02310	1.20890
H	3.84760	0.80060	-0.53090
C	2.85330	-1.62640	0.44770
H	3.59730	-1.78220	-0.34220
H	3.37120	-1.65480	1.41270
H	2.12770	-2.44370	0.41310
H	0.17780	-1.19310	1.00710
O	1.13720	-0.27860	-0.79630
H	-1.44990	-0.20670	1.68340
21			
007 out	0.0000000		
C	0.92660	-4.08450	3.44320
H	1.94480	-4.17650	3.83470
H	0.37990	-5.00770	3.68390
H	0.99150	-3.99290	2.35190
O	0.33510	-2.92960	4.05150
C	-0.31960	1.78290	-0.09720
O	-1.07790	2.26310	-1.14870
H	-0.50320	2.30660	0.83590
C	0.54630	0.78200	-0.14720
C	1.54260	-0.12590	0.35580
C	2.97620	0.32460	0.51660
H	3.18380	1.20820	-0.09420
H	3.16550	0.57370	1.56740
H	3.66230	-0.48300	0.23210
C	1.14450	-1.38100	1.09860
H	1.80000	-2.20970	0.80350
H	1.24990	-1.23810	2.17950
H	0.10560	-1.65180	0.89180
H	-0.55330	-2.81470	3.67440
O	1.05480	-0.09740	-1.08650
H	-0.87410	1.78340	-1.97260
21			
021-in	0.0000000		
C	7.50950	3.68270	-0.73260
H	8.17010	4.49860	-1.02710
H	6.56490	4.10760	-0.39340
H	7.96780	3.14790	0.09960
O	7.28420	2.80920	-1.81610
C	2.19470	2.94720	-1.98470
C	1.47750	4.19040	-1.49510
H	2.09920	4.77770	-0.81820
H	1.20450	4.82610	-2.33790
H	0.56520	3.91800	-0.96370
C	1.35720	2.06790	-2.89790
H	0.50800	1.65690	-2.35160
H	0.96760	2.66180	-3.72530
H	1.91290	1.24000	-3.33390
C	3.46670	2.68440	-1.62210
H	3.97740	3.39870	-0.99150
C	4.30230	1.48190	-2.01660
H	3.68850	0.61320	-2.25400
H	4.87650	1.73350	-2.90960

O	5.19540	1.14740	-0.97160
H	4.71910	0.65300	-0.31790
21			
021 ts	0.0000000		
C	5.11690	3.47040	-1.78960
H	5.20020	4.02260	-2.73980
H	4.07640	3.63540	-1.44290
H	5.78450	3.91880	-1.03880
O	5.35180	2.10940	-1.98690
C	1.62580	2.75520	-1.13160
C	0.85060	3.98410	-0.73070
H	1.04590	4.28860	0.30170
H	1.10160	4.82380	-1.39480
H	-0.22850	3.81240	-0.84820
C	1.41160	2.30960	-2.55490
H	0.33930	2.17570	-2.75160
H	1.76000	3.08980	-3.24620
H	1.92850	1.38180	-2.80800
C	2.46540	2.12840	-0.26460
H	2.59390	2.54410	0.73600
C	3.31820	0.96420	-0.59370
H	2.84530	0.23060	-1.25940
H	4.22240	1.37140	-1.21960
O	3.82780	0.33910	0.57680
H	4.31810	-0.46300	0.31860
21			
021 out	0.0000000		
C	6.35830	2.59230	-1.87100
H	6.67660	3.09230	-0.95250
H	7.13870	2.71660	-2.62970
H	5.43320	3.06620	-2.21970
O	6.15750	1.20390	-1.54650
C	0.88440	2.64810	-1.08830
C	-0.36980	3.27960	-0.55180
H	-0.31090	3.47380	0.52410
H	-0.58680	4.23660	-1.05290
H	-1.24960	2.64260	-0.73300
C	0.89870	2.37310	-2.56820
H	-0.06270	1.95590	-2.89780
H	1.04780	3.29720	-3.14890
H	1.68210	1.67050	-2.86760
C	1.97080	2.38670	-0.25650
H	1.86290	2.62560	0.80340
C	3.19230	1.85130	-0.63060
H	3.44310	1.58640	-1.65340
H	5.87240	0.72980	-2.34730
O	4.16490	1.60570	0.30020
H	4.95080	1.28120	-0.19630
20			
022 in	0.0000000		
C	5.08520	2.92800	-1.60250
H	5.82760	2.99090	-2.40180
H	4.35590	3.73690	-1.72370
H	5.58870	3.04430	-0.63550
O	4.46300	1.64280	-1.71650
C	1.56470	2.75860	-1.14770
C	0.58660	3.83880	-0.75160
H	0.78560	4.23660	0.24820
H	0.62130	4.67170	-1.46750
H	-0.44350	3.45660	-0.77350

C	1.33650	2.17800	-2.52200
H	0.33710	1.72750	-2.58460
H	1.36470	2.97540	-3.27630
H	2.07950	1.43010	-2.80100
C	2.55250	2.36990	-0.32660
H	2.66150	2.84360	0.64790
C	3.58390	1.30640	-0.64160
H	3.10950	0.38000	-0.99200
O	4.30920	1.05820	0.55480
H	4.88370	0.28940	0.39410
20			
finalTSOX0-022-DRY0_1.01_wet.01_957.01			0.0000000
C	5.93680	2.85260	-1.39930
H	6.33230	3.82410	-1.73490
H	5.08750	3.04870	-0.71110
H	6.68500	2.30690	-0.79860
O	5.41880	2.08370	-2.41110
C	1.26780	2.67170	-1.12640
C	0.43360	3.88160	-0.81340
H	0.70990	4.34010	0.14140
H	0.53220	4.64810	-1.59710
H	-0.63630	3.62770	-0.76840
C	0.92510	1.95770	-2.40380
H	-0.09730	1.55210	-2.36270
H	0.95110	2.65010	-3.25740
H	1.59860	1.12880	-2.63370
C	2.28720	2.27070	-0.26950
H	2.44850	2.86150	0.63250
C	3.14470	1.19450	-0.41630
H	3.16610	0.53450	-1.27660
O	4.06640	0.94480	0.57660
H	4.81520	0.45300	0.21600
20			
022 out			0.0000000
C	5.51940	1.60630	-1.04080
H	6.21550	1.39710	-1.85980
H	4.80720	2.37050	-1.38290
H	6.09450	2.03100	-0.20560
O	4.86910	0.38410	-0.68970
C	0.04910	2.98280	-0.99850
C	-1.14700	3.85000	-0.71770
H	-1.21140	4.14890	0.33200
H	-1.11260	4.75290	-1.34190
H	-2.06840	3.31880	-0.99150
C	0.19010	2.55480	-2.43460
H	-0.71520	2.01640	-2.74450
H	0.25690	3.44480	-3.07380
H	1.05390	1.92020	-2.63720
C	0.89840	2.64800	0.00800
H	0.70850	3.01540	1.01540
C	2.08230	1.82760	-0.12740
H	2.32600	1.42120	-1.12360
O	2.83200	1.56370	0.82440
H	4.22980	0.61080	0.01690