

Exploring the Potential for Allergic Contact Dermatitis *via* Computed Heats of Reaction of Haptens with Protein End-groups

Heats of Reaction of Haptens with Protein End-groups by Computation

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Abstract

Unlike human sensitization in allergic contact dermatitis, the primary event in the guinea pig maximization test (GPMT) is not penetration to the viable epidermis as the skin is compromised in both the sensitization and elicitation steps. The primary event is the chemical reaction of a hapten with simple protein end-groups at the recognition site of the major histocompatibility complex, class 2 (MHC II) on the cell surface of the Langerhans cells (LC). This reaction converts a benign LC into an allergen presenting cell that a T(CD4) lymphocyte recognizes as non-self and initiates the sensitizing sequence in the local draining lymph node. The widely used GPMT was selected for the purpose of modelling the sensitizing reaction without the complications of penetration to the viable epidermis.

The compounds selected for study represent the class of electrophilic haptens that, along with their metabolic precursors (prohaptens) comprise the majority of known contact allergens. As the whole MHC II structure has no involvement with the sensitizing reaction beyond the first

few atoms of the end group, methoxide ion and thiomethoxide ion were used to model activated serine and cysteine. In addition, the GPMT has a relatively broad range of error which allows the use of AM1 as an adequate method for modelling the relative heats of reaction in the rate determining step.

The relative reactivity of most of the known electrophilic haptens in the simplest aliphatic and aromatic form are modelled in this study. For many of those that are sensitive to substituent effects, sets of 7–10 substituted analogs were modelled to provide excellent Hammett relations with the heats of reaction. These linear free energy relations allow prediction of ACD potential in new related haptens.

As a final validation of the procedure, a set of compounds scoring non (1), weak (2), moderate (3) and strong (4) as measured in the GPMT are computed for comparison with the test results and found to be in good concordance.

1 Introduction

Allergic contact dermatitis (ACD) is a serious problem in the broad range of products that come into public use and it can

be clearly stated that no drug, pesticide, paint chemical or other commercial or household chemical will long survive with this reactive potential. A case in point is the vaginal fungicide, Chlordantoin, which had no serious effect on the mucous membrane, but was removed from use due to severe contact dermatitis through dermal contact [1, 2]. In the public domain, the first primary event is dermal contact and subsequent penetration of the stratum corneum into the viable epidermis. This is a physical event, well studied in this laboratory [3–6] and elsewhere [7–11] as an independent event. In the case of a prohaptens such as the plant derived catechol, urushiol, a fast intermediate bio-oxidation is required to convert the primary penetrant to a protein reactive hapten. However, the real primary event that initiates the sensitization sequence is the reaction of the hapten with

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Abbreviations: GPMT, Guinea Pig Maximization Test; MHC-II, major histocompatibility complex, Class 2; LC, Langerhans Cells; ACD, allergic contact dermatitis; AM1, Austin Model 1; RHF, restricted Hartree-Fock; Hf, heat of formation; Hrxn, heat of reaction

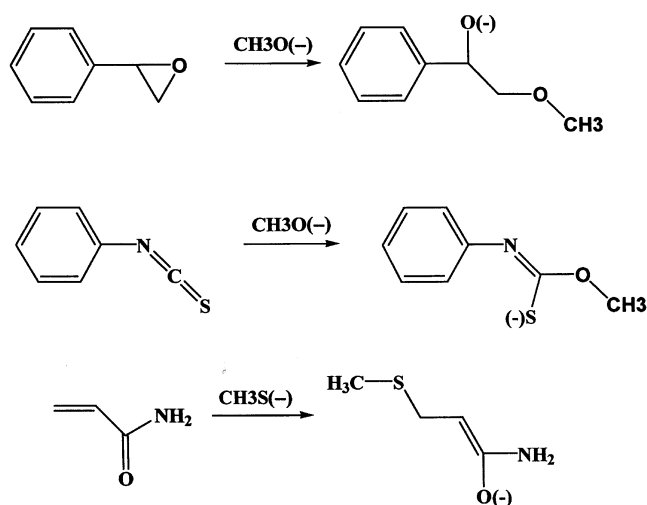


Figure 1. Some examples of model hapten-protein end-group reactions.

nucleophilic protein end-groups on the recognition site of the MHC II molecule located on the cell surface of the Langerhans cell. The sequence is well described and need not be repeated here [12, 13].

Modelling of the ACD sensitization process including stratum corneum penetration has been extensively explored by statistical techniques. Four models have been developed in this laboratory, all with different sets of haptens and prohaptens. The first model was based on a data set of 72 compounds drawn from the literature on human and animal sensitization and had as a goal the simple discrimination of allergens from non-allergens [14, 15]. Based on a rarely used discrimination method [21], two-value regression analysis, the model optimized at $n = 72$ after trials at $n = 24, 48, 72$ and 130. The success of the model in validation studies [15–18] led to publication of an improved model based on the same data set [6]. A second model based on a completely different data set explored the intensity of the ACD response in terms of non (1), weak (2), moderate (3) and strong (4) and found to express the identical factors for dermal penetration [6]. The third model was based exclusively on human data and again expressed the same factors for the primary penetration step [19]. Lastly, a pure GPMT model was developed from data collected by Cronin and Basketter [20] with the interesting result that no statistically significant factors relating to dermal penetration were observed, in agreement with the intent of the test protocol [30, 31].

Hapten reactions with nucleophilic protein end-groups have much in common that simplifies the modelling problem. In a living animal or human, such nucleophiles may be found in the bound state as reactive side-groups of lysine, serine and cysteine on the receptor arm of the major histocompatibility

complex (MHC-II) of the Langerhans cell. These cells derive from stem cells in the bone marrow and the mature cell is broadly distributed in the viable epidermis and lymph nodes [12, 13]. The cell surface is populated with various adhesion molecules and the MHC-II transmembrane protein. As a dendritic cell, it is uniquely constructed for interaction with the T(CD4) lymphocyte when activated by a modifying reaction with an electrophilic hapten. Driven by a chemokine to the paracortical sector of the local draining lymph node, the activated LC (now an allergen presenting cell), binds reversibly with the enormously diverse population of T lymphocytes until a near perfect fit of lowest free energy is achieved. Monoclonal proliferation of the selected T cell is followed by redistribution throughout the body and epidermal layer. A subsequent challenge of the same hapten or cross-related hapten is met with the severe delayed response known clinically as allergic contact dermatitis.

In building a reactivity scale for haptens with protein end-groups, a practical level of computation needs to be selected and carefully assessed. As the response of the GPMT has been assessed by Gad at $\pm$ one level on the 5-level scale from non to severe [22, 23], there is no need for excessive accuracy, though relative performance of the computational scale is important. The semi-empirical quantum mechanical program, AM1, calculates heats of formation directly and heats of reaction indirectly, in addition to other features of electronic interest [24]. In representing the protein end-groups, only the terminal features are needed as the remaining protein is not involved in the reactive step. In addition, it is clear that these highly exothermic reactions must take place in human/animal skin during the brief residence in the viable epidermis, that is, reaction must be faster than transit to the dermis where the unreacted hapten will be cleared through the capillary network. A reasonable assumption is made that these fast reactions at 37°C are thermodynamically controlled and exhibit very low activation energies. This further implies that the rate determining step is the adductive addition of the end group to form an anionic intermediate with all subsequent steps being fast. Computation of relative reactivity depends simply on computing the heats of formation of the two reactants and that of the first intermediate. Some reaction examples are shown in Figure 1.

While AM1 is not known for high accuracy in computing heats of formation [24], a related study of quinone haptens showed exceptional sensitivity to structural changes (substituents) and perfect concordance with the synthetic literature [25]. The haptens in this study do not have the relational similarity of a set of quinones, but the key atoms undergoing the major change in these reactions display parallel features and similar mechanisms.

This study covers nearly all of the known hapten classes as reported by the developers of DEREK [26], modelled in the

simplest aliphatic and/or aromatic form. In addition, many of those expected to vary significantly with structural changes are modelled with sets of 7–10 substituents to provide predictive values for untested compounds.

Finally, a set of 21 unrelated compounds with experimental GPMT ratings from non-strong (1–4) are modelled to compare the computational heats of reaction with the clinical result.

2 Computational Procedures

All computations were performed on a TriStar Pentium/150 MHz computer using the AM1 program [24] in Hyperchem 4.5 [27]. The nucleophiles, MeO(–) and MeS(–) were selected to represent activated serine and cysteine and to explore the difference between first and second row reactants. In earlier studies, some work was also done with methylamine which in the neutral form represents activated lysine (protonated at pH 7) [28]. For haptens explored for substituent effects, ten computable groups were selected covering the electronic range from strong electron donation to strong electron withdrawal (Tables 2–8). In all cases, the first intermediate of the hapten end-group reaction is modelled and the heat of reaction is computed as shown in equation 1. All subsequent steps (protonation, rearrangement) are reasonably assumed to be faster than the initial adduction step, though there may be some exceptions. All computations are reported in kcal/mol and are easily verified with any version of AM1.

$$\text{Hrxn} = \text{Hf}(\text{adduct}) - \text{Hf}(\text{reactants})[\text{hapten} + \text{MeX}(-)] \quad (1)$$

3 Results

3.1 Reactivity of Some Known Haptens and a Few Related Compounds

Before dealing with specific classes, it seems relevant to examine the computed reactivities of some well studied haptens and a few closely related compounds. This immediately provides a scale of reactivity for the scrutiny of experienced organic chemists and perhaps, forms the basis for a qualitative validation of the concept. Compounds severely out of line would be spotted by anyone with extensive experience in synthesis. The haptens in Table 1 were drawn from earlier studies [6, 14, 15] and from the compilation by Cronin and Basketter [20]. Some undocumented electrophiles capable of expressing similar reactivity are included for comparison.

The observed collinearity between the heats of reaction of methoxide and thiomethoxide (Eqs. 2 and 3) indicate that

either anion can effectively model the relative reactivities of the haptens. However, there is significant scatter among the many reaction classes and the indicator variable, IArX, clearly shows the different scaling of the aromatic halides. In the very tight relation of Eq. 3, only one reaction class (quinone haptens) is represented [25]. Thus, the relative heats of reaction are *approximately* independent of the nucleo-

Table 1. Computational heats of reaction of simple haptens with methoxide and thiomethoxide ions. Heats are in kcal/mol

Hapten	Hrxn(MeO –)	Hrxn(MeS –)
Acetone	–19.95, –20.00	
Benzaldehyde	–24.45, –24.46	
Benzenesulfonyl Azide	–24.63	
Acetophenone	–25.69	
Acetaldehyde	–25.74, –25.75	
Propiolactam	–27.90	
Methanesulfonyl Azide	–31.37	
Butyrolactone	–31.45	
Acetyl Azide	–31.60	
Benzoyl Azide	–31.99	
Benzoyl Fluoride	–32.68	
Biacetyl	–32.91, –32.94	
Acetyl Fluoride	–35.51	
Dibenzoyl	–35.69, –35.82	
Acrylamide	–36.65	–23.54
Methyl Vinyl Ketone	–38.03	–24.76
Methyl Isothiocyanate	–38.06, –38.07	–26.25, –26.33
Methyl Thiolacetate	–39.31	
Acrolein	–39.64	–26.19
Propiolactone (adduct)	–40.60, –40.65	
Acetic Anhydride	–41.01, –41.05	
Propylene Oxide	–42.30, –42.31	
Phenyl Benzoate	–42.32, –42.35	
Phenyl Isothiocyanate	–44.92, –45.08	–30.23, –30.25
Styrene Oxide	–45.14, –45.15	
p-Benzoquinone	–45.91	–33.22
Methyl Isocyanate	–46.27	–25.66, –25.67
Phenyl Acetate	–46.32, –46.48	
o-Benzoquinone (4-pos.)	–47.66	
Acetyl Chloride	–50.92, –50.93	
Phenyl Thiolbenzoate ¹	–51.87	
Phenyl Methyl Carbonate	–52.21	
o-Benzoquinone (3-pos.)	–54.53	
Methanesulfonyl Chloride	–56.08	
Phenyl Isocyanate	–60.12	–35.15, –35.16
Benzenesulfonyl Chloride	–68.17	
Propiolactone (ring opened)	–73.84	
Benzoic Anhydride ²	–113.41, –116.20	

1. The parent compound converged with difficulty and variability. The lowest heat of formation of several starting geometries was used to estimate the heat of reaction.

2. The intermediate of this hapten converged with great difficulty and may have substantial error.

phile, supporting the use of methoxide ion for modelling the majority of hapten reactions.

$$\text{Hrxn}[\text{MeO}(-)] = 0.981\text{Hrxn}[\text{MeS}(-)] - 5.62\text{IArX} - 14.76$$

$$T = 12.97 \quad -2.98 \quad (2)$$

$$n = 15 \quad r = 0.969 \quad F = 92$$

$$\text{Hrxn}[\text{MeO}(-)] = 1.092\text{Hrxn}[\text{MeS}(-)] - 10.71$$

$$T = 71.80 \quad (3)$$

$$n = 28 \quad r = 0.998 \quad F = 5155$$

All of the haptens and related compounds in Table 1 have intermediates with highly localized negative charge on oxygen or sulfur *via* similar nucleophilic addition reactions. These compounds and the substituted analogs discussed in the following section on response to substitution can be reasonably expected to fall on the same scale of reactivity.

3.2 Treatment of Hapten Reactions Having a Strong Response to Substitution

Many of the simple haptens of Table 1 have reactive centers sensitive to olefinic or aromatic substitution, such that the full range of structure might cover two or even three degrees of severity in the GPMT with some members showing no ACD response. This can only be explored by computing a full range of substitution for comparison with the scale for simple haptens. In the case of quinone haptens, a selection of ten easily computed substituents covering a full range from strong electron donation to strong electron withdrawal was used to obtain a Hammett rho-sigma relation from which new values could be computed [25]. In this particular case, the heats of reaction ranged from -48 to -82 kcal/mol, all of which reflect strong ACD potential.

With the exception of the conjugated olefins where the full set contained only 7 members, the remainder of the sets were computed with 9–10 substituents. Tables 2–8 show the individual computations and the equations expressing the dependence of the heats of reaction on the experimental Hammett sigma constants. A few statistical outliers were observed and deleted from the sets to obtain a sharper relation, though none were serious enough to obscure the trend of the dependence.

The regression slopes from Tables 2–8 vary with proximity of the reaction site to the substituted ring or olefin and should be useful for estimating the substituent response of new systems, certainly well enough to form a strong opinion of the ACD reactivity of an untested compound (Table 9).

Table 2. Electronic response of substituted 1,4-benzoquinones reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -) ¹	Sigma
Me2N	-48.26	-0.83
MeO	-50.65	-0.27
Me	-47.86	-0.17
H	-45.91	0.00
F	-55.69	0.06
Cl	-53.05	0.23
CF3	-67.48	0.54
CN	-66.62	0.66
COOCH3	-67.46	0.45
NO2	-82.28	0.78

1. Heat of reaction at the most reactive position [25].

Spread in heats of reaction = 34.02 kcal/mol

Hrxn(MeO-) = -29.4 Sigma(p) - 52.2

T = -5.92

n = 9 r = 0.913 s = 5.25 F = 35.0

1 outlier (Me2N).

Table 3. Conjugated vinyl haptens reacting with methoxide and thiomethoxide ion. Computational heats are in kcal/mol

Hapten	Hrxn(MeO -)	Hrxn(MeS -)
Acrylamide	-36.65, -36.66	-23.54, -23.55
Methyl Vinyl Ketone	-38.03, -38.04	-24.76, -24.77
Acrolein	-39.64	-26.19, -26.21
Methyl Acrylate	-41.43, -41.44	-26.93, -26.95
Acrylic Acid	-42.44, -42.45	-28.04, -28.06
Nitroethylene	-59.55	-43.26, -43.28
Methyl Vinyl Sulfone	-61.94, -62.11	-46.50, -46.68

Spread in heats of reaction (MeO -) = 25.46.

Spread in heats of reaction (MeS -) = 23.14.

Hrxn(MeO-) = -62.8 Sigma(p) - 12.1

T = -6.89

n = 7 r = 0.951 s = 3.56 F = 47.5

Table 4. Electronic response of 4-substituted benzaldehydes reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -)	Sigma
Me2N	-28.72	-0.83
MeO	-29.82	-0.27
Me	-29.63	-0.17
H	-24.46	0.00
F	-33.78	0.06
Cl	-34.29	0.23
CF3	-39.57	0.54
CN	-38.68	0.66
COOCH3	-37.46	0.45
NO2	-44.91	0.78

Spread in heats of reaction = 16.19 kcal/mol.

Hrxn(MeO-) = -12.9 Sigma(p) - 32.3

T = -8.56

n = 8 r = 0.961 s = 1.54 F = 73.3

2 outliers (H, NO2).

Table 5. Electronic response of 4-substituted phenyl isocyanates reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -)	Sigma
Me2N	-55.84	-0.83
MeO	-60.04	-0.27
Me	-60.15	-0.17
H	-60.13	0.00
F	-64.60	0.06
Cl	-65.42	0.23
CF3	-72.35	0.54
CN	-71.61	0.66
COOCH3	-71.12	0.45
NO2	-80.16	0.78

Spread in heats of reaction = 24.32 kcal/mol.

Hrxn(MeO-) = -12.3 Sigma(p) -63.7

T = -7.88

n = 9 r = 0.948 s = 2.05 F = 62.0

1 outlier (NO2).

Table 6. Electronic response of 4-substituted phenyl isothiocyanates reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -)	Sigma
Me2N	-41.27	-0.83
MeO	-44.86	-0.27
Me	-44.77	-0.17
H	-45.08	0.00
F	-48.60	0.06
Cl	-49.08	0.23
CF3	-54.43	0.54
CN	-53.72	0.66
NO2	-60.35	0.78

Spread in heats of reaction = 19.08 kcal/mol.

Hrxn(MeO-) = -9.3 Sigma(p) -47.5

T = -7.71

n = 8 r = 0.953 s = 1.51 F = 59.5

1 outlier (NO2).

Table 7. Electronic response of 4-substituted benzoyl fluorides reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -)	Sigma
Me2N	-35.04	-0.83
MeO	-36.99	-0.27
Me	-37.00	-0.17
H	-32.68	0.00
F	-41.17	0.06
Cl	-41.67	0.23
CF3	-47.00	0.54
CN	-45.85	0.66
NO2	-52.08	0.78

Spread in heats of reaction = 17.04 kcal/mol.

Hrxn(MeO-) = -10.1 Sigma(p) -40.8

T = -6.58

n = 8 r = 0.937 s = 2.21 F = 43.3

1 outlier (H).

Table 8. Electronic response of 4-substituted styrene oxides reacting with methoxide ion. Computational heats are in kcal/mol

Substituent	Hrxn(MeO -)	Sigma
Me2N	-48.97	-0.83
MeO	-51.13	-0.27
Me	-49.13	-0.17
H	-45.15	0.00
F	-54.70	0.06
Cl	-55.11	0.23
CF3	-58.35	0.54
CN	-57.57	0.66
NO2	-63.35	0.78

Spread in heats of reaction = 14.38 kcal/mol.

Hrxn(MeO-) = -8.40 Sigma(p) -53.7

T = -5.59

n = 8 r = 0.916 s = 2.16 F = 31.2

1 outlier (H).

Table 9. Comparative electronic response of haptens to substituent proximity. Computational heats are in kcal/mol

Hapten series	Data span	Rho
Conjugated Olefins	25.46	-62.8
Quinones ¹	34.02	-29.4
Phenyl Isocyanates	24.32	-12.3
Benzaldehydes	16.19	-11.0
Benzoyl Fluorides	17.04	-10.1
Phenyl Isothiocyanates	19.08	-9.3
Styrene Oxides	14.38	-8.4

1. Most reactive position [25].

3.3 Treating Hapten Reactions of Reactive Aromatic Halides

The haptens presented in sections 3.1 and 3.2 react by similar mechanisms to produce anionic intermediates with similar charge localization. Intermediates are also formed in the nucleophilic attack of MeX(-) at the carbon atom of a displaceable aromatic halide, but the charge is now delocalized over several ring atoms. This is a substantially different mechanism and the scaling relative to the localized intermediates of 3.1 and 3.2 is unknown. Thus, it seems best to consider these reactions on a relative scale of their own until the break-point or region between ACD reactivity and non-allergenic structures is better known.

Table 10 is a sampling of Table 1 from the book by Dupuis and Benezra [29], where they describe the ACD response of some substituted halobenzenes. Qualitatively, it can be seen that 2-3 properly placed nitro groups are required to promote sufficient reactivity to induce ACD sensitivity. And yet, the least reactive compound, hexachlorobenzene, reacts at ambient temperature with two moles of MeS(-) in methanol to form the 1,4-di-[methylthio]aromatic [author's experience]. In Table 11, the 10 kcal/mol region separating the two

classes of reactivity is clearly seen in the break between 1-fluoro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene.

Table 12 shows computations of 5- and 6-member heterocyclic halides, most of which are clearly less reactive than the nitroaryl halides of Table 11. This is a large unexplored area of computation with very little experimental support from the GPMT and other methods of ACD sensitization. Although

Table 10. Qualitative allergic response to some electrophilic aromatic halides¹

Aromatic halide	Allergic response
1-Chloro-2,4-dinitrobenzene (also fluoro, bromo, iodo)	positive
1,4-Dichloro-2,6-dinitrobenzene	positive
1,3-Dichloro-4,6-dinitrobenzene	positive
1-Chloro-2,4,6-trinitrobenzene	positive
1,3-Dichloro-5-nitrobenzene	negative
1,4-Dichloro-2-nitrobenzene	negative
1,2-Dichloro-4-nitrobenzene	negative
4-Chloro-1-nitrobenzene	negative
Hexachlorobenzene	negative

1. Abstracted from Table 1 of reference [29].

Table 11. Computed heats of reaction of thiomethoxide and methoxide ion with electrophilic aromatic halides. Computational heats are in kcal/mol

Aromatic halide	Hrxn(MeS -)	Hrxn(MeO -)
1,2,3-Trichloro-4,6-dinitrobenzene ¹	-60.91	-79.86
1-Chloro-2,4-dinitrobenzene	-53.04, -53.06	-75.16, -75.20
1-Fluoro-2,4-dinitrobenzene	-47.97	
1,2-Dichloro-4-nitrobenzene	-37.61	
1,4-Dichloro-2-nitrobenzene	-35.11	
1-Chloro-4-nitrobenzene	-33.98	
1-Chloro-2-nitrobenzene	-29.20	

1. Commercial fungicide.

Table 12. Computed heats of reaction of methoxide and thiomethoxide ion with electrophilic heterocyclic halides. Computational heats are in kcal/mol

Aromatic halide	Hrxn(MeO -)	Hrxn(MeS -)
1-Chloro-2,4-dinitrobenzene	-75.16, -75.20	-53.04, -53.06
1-Fluoro-2,4-dinitrobenzene	-47.97	
2-Chloro-3,5-dinitrofurane	-95.49	
2-Chloro-5-nitrofurane	-68.75	
2-Chloro-4-nitrofurane	-52.68	
3-Chloro-5-nitrofurane	+10.63	
2-Fluoropyrimidine	-34.80	-12.96
4-Fluoropyrimidine	-39.73, -39.78	-14.80
2-Chloropyrimidine	-34.09, -34.10	-19.29
4-Chloropyrimidine	-39.41, -39.42	-21.92
2-Fluorotriazine	-45.75, -45.76	-22.08, -22.15
2-Chlorotriazine	-43.33, -43.34	-28.53, -28.58

the present treatment is structurally light, there seems little point in extensive computations prior to significant clinical reports.

3.4 Computing the Guinea Pig Maximization Test

In the guinea pig maximization test, the skin is compromised in both the sensitization protocol (intradermal injection) and in the challenge protocol (irritation) [30, 31]. In addition, the guinea pig is sensitized at the maximum dose just below the level of predetermined irritation, hence the concept of a maximization test. These procedures provide a measure of public safety by obviating the modifying effect of the stratum corneum barrier. In one unpublished attempt to model the GPMT, there was no evidence of the skin penetration factors observed in the other models [6, 14, 15]. By eliminating the skin barrier, the GPMT provides a clinical animal test to measure the potential of a compound for sensitizing the mammalian immune system. While perfect concordance with human sensitization is not achieved, the GPMT errs on the side of public safety as it was designed to do. Now here is the interesting part. The computational method of this study has exactly the same goal as the GPMT in attempting to develop a scale of protein end-group reactivity that will express the same potential for mammalian ACD sensitization. It is clearly important, therefore, to compare known GPMT responses with the corresponding computational heats of reaction.

Table 13 lists a sampling of GPMT responses developed in a single laboratory [20]. The preference for single laboratory data is clear from the study of Gad, who determined that the variance in lab GPMT's was roughly $\pm$ one response level [22]. As both ratings and experimental technique may vary from one lab to another, data from a single source provides a better basis for comparison with computation. The data in Table 13 may be summarized as the range of the computed heats and the average values as follows.

Class 1: non or very weak—None of the six compounds in this class contain groups that are reactive toward activated end-groups in the absence of enzymic catalysis. While many can form adducts with methoxide ion, these adducts are reversible and do not lead to products.

Class 2: weak—This class contains only four compounds and may vary somewhat as additional cases are studied. Range = -19.90 to -33.75 kcal/mol/Average = -27.22 kcal/mol.

Class 3: moderate—Range = -26.68 to -48.37 kcal/mol/Average = -39.94 kcal/mol.

Class 4: strong to severe—Range = -36.59 to -60.13 kcal/mol/Average = -44.47 kcal/mol.

Table 13. Haptens measured in a single laboratory by the guinea pig maximization test [20]

Hapten	GPMT rating	Hrxn(MeO —)
2-Butoxyethanol	1	0
Ethyl lactate ¹	1	0
N-Octylpyrrolidone ¹	1	0
2-Piperidone ¹	1	0
N-Acetyl caprolactam ¹	1	0
Salicylamide ¹	1	0
2-Hexylcyclopentanone	2	−19.90
1-Carvone	2	−32.15
4-t-Butyl phenylacetone	2	−23.08
3,4-[Methylenedioxy]benzaldehyde	2	−33.75
Citral	3	−26.68
2-Pentylcyclopent-2-enone	3	−40.24
Methyl glyoxalate	3	−47.68
Methylene bis-acrylamide	3	−48.37
4-Bromophenacyl bromide	3	−36.72
Phenyl isothiocyanate	4	−45.08
Phenyl isocyanate	4	−60.13
Allantolactone	4	−39.22
Diethyl fumarate	4	−43.66
Cinnamaldehyde	4	−36.59
Glyoxal	4	−42.13

1. Reversible reactivity only.

This small study was performed as an early check on the computational method and is not intended to define a computational GPMT. Many more hapten-end group reactions will be required to meet this goal. Nevertheless, the relation is remarkably strong at this level (equation 4).

$$\text{GPMT} = -0.0551\text{Hrxn} + 1.013$$

$$T = -9.13 \quad (4)$$

$$n = 21 \text{ (no outliers)} \quad r^2 = 0.814 \quad F = 83.35$$

4 Discussion

4.1 Computational Objectives

In developing a rank model expressing the range of ACD response as non(1), weak(2), moderate(3), strong(4), there was both success and failure in our use of dichotomous descriptors to express protein reactivity [6]. Success came in the form of revealing exactly the same skin penetration terms as our earlier discriminant model (non and active) with a completely different set of compounds. But when one examines the plot of input ranks against the predicted values, it becomes clear that the model is compressed. The mean values of estimated 1's (1.30) and 4's (3.77) cover a span of only 2.47 rather than 3. In brief, the class 1 non's are overpredicted and the class 4 strong's are underpredicted, a result of forcing 2-value descriptors into a 1–4 range of responses. In order to adequately model a range of ACD

response, we must have quantitatively graded protein reactivity descriptors.

The judgement that electrophilic haptens react with protein end-groups in a fast, exothermic reaction is based on the low residence time of most chemicals in transit across the viable epidermis [8]. This assumption was verified in the first few calculations and in a previous computational study of reactive quinone haptens [25]. There are clearly two kinetic processes in operation, one involving the rate of transdermal transport and the other the rate of hapten reactivity with the protein end-groups of the MHC II molecules on the LC surface. By recognizing these reactions as exothermic, low activation energy processes, we have the luxury of dealing with the exotherm of the rate-determining step to the first intermediate.

In considering the span of molecular complexity, especially in modelling the flexible intermediates, AM1 proved to be an ideal choice. While most of the haptens converged with ease, even from Dreiding structures, the intermediate adducts were frequently difficult even after molecular modelling. Many were highly flexible and tended to oscillate from low to high energies while slowly converging over long hours. The AM1 program was able to converge nearly all of the desired structures while also displaying very good response to computational substituent effects. The similarity of the hapten/end-group reactions is clearly a factor in the computational results.

Finally, it will be noted from entries in Tables 1, 3, 11 and 12 that duplicate runs were made to determine repeatability and in all cases of difficult conversion. Nearly all are closely repeated. Only two exceptions with probable error are noted in Table 1, phenyl thiolbenzoate and benzoic anhydride. Explanations are cited in the footnotes.

4.2 Simple Electrophilic Haptens

In the kinetic sense, potential haptens can be either too reactive or not reactive enough. If too reactive, the hapten may never reach the viable epidermis, reacting in the SC or with water at the interface of the living tissue. The hapten, methacrylamide, is an interesting example of high protein reactivity as only 40–75% penetrates whole skin in a transdermal experiment on rabbits [32].

The haptens and related compounds in Table 1 cover a majority of the electrophilic types in the DEREK knowledge based system [26]. The first compound, acetone, is not an ACD hapten and was computed to represent the class of simple ketones such as cyclohexanone. The computed exotherm with MeO(—) is only 4.5 kcal/mol below that of the hapten, benzaldehyde. In addition to relatively low reactivity, acetone is known to destroy the integrity of the SC,

removing the rate-determining barrier to transport across whole skin [33]. It is also interesting to compare the reactivity of acetone with that of biacetyl, where the carbonyl groups are mutually activating and also that of methyl vinyl ketone which reacts at the beta-vinyl carbon. Propiolactam was modelled to represent the class of penicillins, some of which express an ACD response [34]. The related propiolactone is a known carcinogen reactive with nucleotide bases. It is not known as a hapten, though human contact is rare. The lactone is interesting for two potential modes of nucleophilic attack, namely, carbonyl adduction and beta-ring opening. Both are highly exothermic with the synthetically observed ring-opening strongly favored. Ortho-benzoquinone represents the reactive metabolite substructure of the prohaptens, urushiol (poison ivy, oak, sumac). It is somewhat more reactive than p-benzoquinone with the 3-position more reactive than the 4 [25]. Lastly, one of the surprises of this study was the very high reactivity of phenyl methyl carbonate which exceeds that of the known hapten, phenyl benzoate, by 10 kcal/mol.

In general, there are few surprises in Table 1, certainly none that would upset a chemist skilled in organic reactions. It must be appreciated, however, that while Table 1 samples most of the known electrophilic haptens, it does so only in their simplest form. Many of these will be sensitive to structural changes, particularly changes that increase or reduce electropositivity at the reaction site. It is not uncommon for a single class of electrophiles to encompass the entire range of ACD response from non to strong. For this reason, it is obligatory to study a full range of substituent effects on many of these classes.

4.3 Electronic Effects in Haptens

Seven classes of haptens were selected for studies of response to a full range of substituents with the expectation that the computed heats of reaction would correlate well enough with Hammett sigma values to provide predictive equations. Tables 2–8 display the results for 1,4-benzoquinones, conjugated vinyl haptens, benzaldehydes, phenyl isocyanates, phenyl isothiocyanates, benzoyl fluorides and styrene oxides. These examples cover a broad range of structures where the reactive site is electronically modified by conjugation with substituents.

The reactivity of 1,4-benzoquinones is complicated by the fact that different types of substituents dictate different positions for ring attack by nucleophiles, including displacement of the substituent itself. In an earlier study, it was found that near perfect Hammett relations were obtained for specific attacks computed for the 3- and 5-positions [25]. While this is good computational chemistry, it does not model the reality of the position dependant attacks dictated by the substituents. In Table 2, the computed heats for reaction with methoxide ion are those of the most reactive position. As a

result of positional variance, the Hammett relation is much weaker and with one outlier (Me_2N), but still useful for estimating the reactivity of new quinones.

The conjugated vinyl haptens in Table 3 have the strongest observed response due to direct interaction of the electrophilic site with the substituent over a single double bond. The Hammett relation is quite good and the very large rho value (response slope) provides at least a qualitative insight to the fact that vinyl haptens cover the full range of ACD response from non to strong [35]. Acrylic acid was computed in the neutral acid form, while it would predominantly reside as an anion at physiological pH with much lower reactivity.

From benzaldehydes to benzoyl fluorides (Tables 4–7), the correlations and outliers are similar with rho values from -9.3 to -12.3 . Three have the nitro group as an outlier, two have the parent compound (H) and one (benzaldehydes) has both. The nitro group is a common outlier in basic reactions while the (H) group is anomalous in many cases [36]. Each of the Hammett equations is satisfactory as a means of estimating the heats of reaction of new members.

Not surprisingly, the styrene oxides (Table 8) gave the weakest electronic response as the site of nucleophilic attack has no conjugative link to the aryl ring. The response is not negligible, however, as the data span of 14.38 kcal/mol is certainly large enough to place the 4-dimethylamino and 4-nitrostyrene oxides in different response classes.

Table 9 summarizes the electronic response from very strong (vinyl haptens and quinones) to moderate (next 4) to borderline (styrene oxides) and should provide a useful means of estimating the reactivity of related haptens.

4.4 Reactive Aromatic Halides

In the intermediates of electrophiles studied in Tables 1–9, charge is localized on oxygen or sulfur, either directly or in small O/S substructures. Aromatic halides react to form non-aromatic intermediates where the negative charge is delocalized over a 3-atom segment which may be all-C or mixes of C, N and/or O (heterocycles). This is clearly a different mechanism and one that cannot be expected to scale with the simpler types. To begin the process of rescaling, we need to locate the break point or region between ACD reactive aromatic halides and those with no response. Early work by Dupuis and Benezra provided many powerful insights to the structure-response relation [29]. Among these is the very first table in their book, part of which is detailed in Table 10. The table clearly shows that 2–3 properly placed nitro groups are required to activate the ring halogen for an ACD response, although all of the halides in the table (haptens, non-haptens) are reactive at 25°C with thiomethoxide ion in ethanol. While all may be of adequate reactivity, the structural changes also

vary the rate of transport and residence time in the viable epidermis. Thus, one way of viewing the compounds of Table 10 is one of transport versus reactivity. One class (non-haptens) crosses to the dermis and clears before it reacts, while the other class (haptens) reacts faster than it crosses.

In Table 11, reaction of the activated halobenzenes was studied with thiomethoxide ion as this is the more common nucleophile for such displacements. The 10 kcal/mol gap between 1-fluoro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene clearly separates the two classes. It will be interesting in future studies to locate aryl halides with intermediate reactivity and compare the computations with the GPMT. As many groups other than nitro (CN, MeSO₂, CH=O etc.) can activate ring halogens, an entire field of study is open for both computation and animal testing.

Table 12 explores a select few of the many 5- and 6-ring heterocycles in which the ring halides are activated in part by in-ring N and O atoms. Two of the 2,4-dinitrophenyl halides are included for comparison as these represent tested haptens. In the chlorofurans, it again requires two properly placed nitro groups to bring the compound to the level of hapten reactivity. Moreover, the importance of nitro group position is clearly shown in the almost 80 kcal/mol spread in the 2- and 3-chloro-mono-nitrofurans, the least reactive of which is endothermic from the reactants. None of the pyrimidines or triazines show sufficient reactivity to cause an ACD response. This is surprising as chlorotriazine herbicides such as Atrazine are known to metabolize in part by first conjugating with glutathione [37]. The general field of reactive halo-heterocycles is very large, but there is little reported clinical and animal data to support extensive computation.

4.5 Computation Versus the GPMT

The variance in the GPMT expressed by Gad [22], made it desirable to use data developed in a single lab for comparison with computational reactivity. Fortunately, such data were available in a large study reported by Cronin and Basketter [20]. Moreover, the authors agreed with our shortening of the Magnussen and Kligman scale [23] from five levels to four. In brief, we both combined the strong and extreme response into one level and redefined the first two levels from weak and mild to non/very weak and weak as follows. The percentage ranges represent the number of animals out of 20 that are sensitized:

0–8%	non or very weak
9–28%	weak
29–64%	moderate
65–100%	strong

It should be noted that this method of recording a bioresponse is not typical, as the number of animals sensitized does not

directly address the individual response of any animal, but rather reflects the reliability of sensitization in a randomly selected group of guinea pigs. Intensity is reflected in the reliability measure, but not as a primary observation.

A total of 21 compounds were selected as a test set and the computed reactivities and GPMT ratings are displayed in Table 13. Little can be said about the compounds with GPMT rank = 1. Values can be computed for the association of methoxide ion with ethyl lactate and the 4 amides, but none are expected to consummate the cleavage outside of an enzyme reaction site. The other classes all compute with both the expected overlap and an increasing average heat of reaction as described in 3.4. The observed overlap in values is no greater than observed in our earlier statistical studies and each computation certainly falls within the variance described by Gad [22]. This is a very small test of computation *versus* the GPMT and much more work would be needed to reach any degree of credibility. However, this is a very good start and should provide a useful platform for anyone interested in the computational approach to ACD.

5 General Conclusions

Several important conclusions may be drawn from the results of this study.

1. The excellent concordance of the hapten computational values with the GPMT ratings strongly suggests a direct relation of the ratings with chemical reactivity.
2. The assumption of a fast, early transition state reaction of a hapten with a protein end-group is supported. The high rates of these reactions support the computation of the exotherm from reactants to the first intermediate as a measure of relative reactivity.
3. AM1 provides an adequate computation of the relative heats of reaction of the rate-determining step, with the understanding that reactive aromatic halides are on a different scale than ordinary electrophiles.
4. The lack of perfect concordance between the GPMT and human sensitization is largely due to trans-dermal penetration and/or preempting reactivity in the human SC.

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Dedication

To my friend and colleague, Claude Benezra (deceased), without whom this study would not have been attempted.

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