the solution with sodium hydroxide. HPLC analysis showed loss of both the sensitizer and the cation[23] and the formation of one major new product, which, on the basis of LC/MS evidence, appeared to be an adduct (III) resulting from addition of radical II to the sensitizer (Scheme I). Isolation of the adduct by chromatography on a preparative reverse-phase column confirmed this assignment.[24,25] An additional minor product is also observed by HPLC; LC/MS data is consistent with the radical dimer.

The above results provide conclusive evidence for the electron transfer quenching of triplet 1-methoxynaphthalene by cation I (Scheme I). On the basis of the known oxidation potentials of 0.16 and 1.38 eV for the (4-methoxyphenyl)phenylmethyl radical[26] and 1-methoxynaphthalene,[27] respectively, and the triplet energy for the latter (59.7 kcal/mol),[28] we estimate that this process should be exothermic by ~31 kcal/mol.[29] The relatively efficient formation of separated radicals and radical cations is consistent with the triplet nature of the initial radical/radical ion pair, which allows cage except to compete favorably with back electron transfer. The amount of irreversible cation depletion (~30%) observed in the transient experiments demonstrates that radical/radical cation coupling competes relatively efficiently with back electron transfer, although the latter step is also highly exothermic. Further experiments aimed at measuring the quantum yields for adduct formation and investigating the generality of both the electron transfer reaction and the coupling of the radical and radical cation for a variety of sensitizers and cations are in progress. The variations in the forward and back electron transfer depending on the redox properties of the sensitizer and the cation are of particular interest.

(23) Neutralization of an unirradiated sample of the cation with an equimolar amount of sodium hydroxide gives clean conversion to the corresponding trifluoromethyl ethyl ether.
(24) Physical data for the adduct was consistent with the proposed structure.
(25) The same product can be generated in a thermal reaction between the cation and 1-methoxynaphthalene when the sensitizer and cation are present in much higher concentrations (>0.05 M); an analogous reaction has been reported between the dianisylethyl cation and 1,2,4-trimethoxybenzene.[12]
(26) Private communication: D. D. M. Wayner.
(27) Mattes, S. L.; Farid, S. *Org. Photochem.* **1983**, *6*, 233-326.
(28) Murov, S. L. *Handbook of Photochemistry*; Marcel Dekker: New York, 1973; p 29.
(29) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.

Prediction by AM1 of More Reasonable Geometries for 1,2-Diphenylcycloalkenes Than by Other Semiempirical MO Methods

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In a recent publication,[1] we discussed the geometric and electronic structures of 1,2-diphenylcycloalkenes with $n = 0$–4, where $n = 0$ refers to *cis*-stilbene (see Figure 1).

In that paper, we stated that none of the frequently successfully applied semiempirical MO methods such as MNDO[2] or MINDO[3] were able to predict a reasonable structure for these sterically hindered molecules (see Table I). The steric hindrance caused primarily by H–H interactions completely overrides the π-interaction that tends to stabilize more planar structures of the conjugated system. In contrast, force-field methods such as

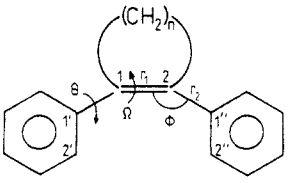

Figure 1.

Table I. Optimized Geometries Derived from Three Different Semiempirical MO Methods and from the Force-Field Method QCFF/PI[a]

n	method	r_1	r_2	ϕ, deg	θ, deg	Ω, deg
0	exptl	1.34	1.49	129	43	
	QCFF/PI	1.35	1.49	127	35	9
	MINDO/3	1.34	1.50	135	90	0
	MNDO	1.35	1.48	129	75	0
	AM1	1.34	1.46	128	47	1
1	exptl					
	QCFF/PI	1.34	1.42	151	7	4
	MINDO/3	1.35	1.48	152	79	1.5
	MNDO	1.34	1.44	152	26	2.7
	AM1	1.33	1.42	152	2	0.3
2	exptl	1.35	1.47	137	16/26	10
	QCFF/PI	1.36	1.46	136	23	9
	MINDO/3	1.38	1.50	138	83	1.5
	MNDO	1.38	1.47	136	65	1.5
	AM1	1.38	1.44	138	27	1.7
3	exptl	1.34	1.48	125	44/48	8
	QCFF/PI	1.36	1.49	128	36	10
	MINDO/3	1.38	1.51	130	90	1
	MNDO	1.36	1.48	128	84	0.5
	AM1	1.36	1.45	129	50	2
4	exptl	1.33	1.49	125	40/55	7
	QCFF/PI	1.36	1.51	122	50	10
	MINDO/3	1.38	1.53	126	90	0.9
	MNDO	1.36	1.50	123	88	0
	AM1	1.33	1.47	124	63	2.7

[a] The experimental geometries are the same as those discussed in ref 6.

MMP2[4] or QCFF/PI[5] provide structures that are in good agreement with the results derived from a variety of experimental methods.[1] We now have applied AM1[6] to the same problem and found that the torsion angles Ω and θ predicted by this method are in much better accordance with those obtained from force-field methods. The main change in AM1 compared to some earlier semiempirical methods is the reparametrization of the nuclear repulsion integrals.[7]

Examining the results in somewhat more detail, we find two trends that are worth mentioning (see Table I): (i) The angle Ω describing the torsion of the central double bond is predicted as fairly rigid in AM1. The force-field methods predict a somewhat higher flexibility, in better agreement with the experimental findings. (ii) The length of the single bond that connects the phenyl rings to the double bond is predicted as a little too short by AM1. MNDO seems to yield the best average results with respect to this bond length.

In comparing theoretical and experimental values of the torsion angle θ, we have to be aware that the torsional potential is flat and very anharmonic.[1] The thermal average of θ is therefore always larger than the value corresponding to the minimum of the potential. Since it is this latter value that is obtained from calculations, we expect the theoretical values to be somewhat smaller than the observed ones.[8] This is the case with the

(1) Hohlneicher, G.; Müller, M.; Demmer, M.; Lex, J.; Penn, J. H.; Gann, L. X.; Loesel, P. D. *J. Am. Chem. Soc.* **1988**, *110*, 4483.
(2) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899, 4907.
(3) Bingham, R. C.; Dewar, M. J. S.; Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1285.
(4) Sprague, J. T.; Tai, J. C.; Yuh, Y.; Allinger, N. L. *J. Comput. Chem.* **1987**, *8*, 581.
(5) Warshel, A.; Karplus, M. *J. Am. Chem. Soc.* **1972**, *94*, 5613.
(6) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(7) As noticed also by other authors, the formula given in ref 6 for the repulsion integrals does not agree with the formula actually used in the AM1 program.
(8) Warshel, A.; Huler, E.; Rabinovich, D.; Shakked, Z. *J. Mol. Struct.* **1974**, *23*, 175.

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force-field methods but not with AM1. The latter method still seems to overstimulate to some extent steric interaction.

In conclusion, we recommend AM1 as the most reliable semiempirical MO method presently available for the structure optimization of sterically hindered hydrocarbons that contain a weakly coupled π-system.

Registry No. *cis*-Stilbene, 645-49-8; 1,2-diphenylcyclopropene, 24168-52-3; 1,2-diphenylcyclobutene, 3306-02-3; 1,2-diphenylcyclopentene, 1485-98-9; 1,2-diphenylcyclohexene, 41317-87-7.

Bait and Switch Strategy for Obtaining Catalytic Antibodies with Acyl-Transfer Capabilities

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Monoclonal antibodies have been shown to catalyze a variety of acyl-transfer reactions[1] by utilizing haptenic transition-state models.[2] In order for the scope and capabilities of these hydrolytic abzymes to be expanded, new strategies must be developed for eliciting catalytic activity in the combining sites of antibodies. Recent reports have focused attention on the modification of an antibody's binding pocket through either semisynthetic methods[3] or site-directed mutagenesis.[4] However, their generality may be reduced because of the lack of available structural data for catalytic antibodies. We felt that a process that could induce catalytically active groups de novo from our antigen might prove more advantageous because one can harness the vast variability of the immune response, via the somatic mutation process, to perform "in vivo" mutagenesis. Herein we report a tactic that elicits an amino acid (or acids) within the antibody's binding pocket to assist in an acyl-transfer reaction by a methodology we have previously termed "bait and switch" catalysis.[2]

Our plan involved the placement of a point charge within our antigen **1a** (Figure 1) in close proximity to the acyl moiety we wished to hydrolyze. The antibodies raised to this hapten should possess amino acid residue (or residues) at the binding site having a charge complementary to this haptenic charge.[5] In addition, the *N*-methylpyridinium salt **1a** will present to the antibody a hydroxylic group having a tetrahedral geometry that will serve as a representation of the acyl-transfer transition state. We intentionally left this position uncharged so that there would be no additional electrostatic effects. The benzoate substrate **3** (Figure 1) corresponding to hapten **1a** has similar steric dimensions (determined from MM2 calculations), but lacks the positive charge. Hence, the amino acid at the antibody binding site will be freed from ion-pair formation and can now serve as a potential general acid/base or nucleophilic catalyst. The pyridine hapten **2** will function as a control, since it is structurally identical with **1a**, but lacks a charge at physiological pH. Charge complementarity by Schultz et al. and Sugasawara has been previously employed to abstract a substrate proton in an antibody-catalyzed β-elimination reaction, although no comparison was made to a neutral hapten.[5d]

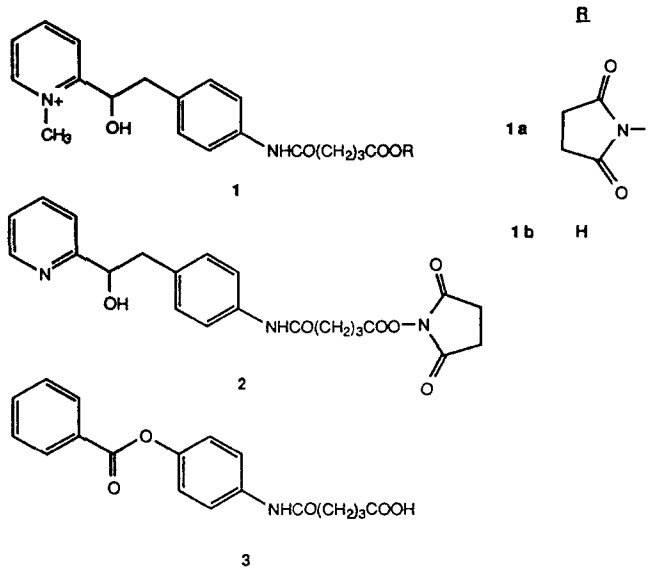

Figure 1. Structures of antigens (**1a**, **2**), inhibitor (**1b**), and substrate (**3**).

Hapten **1a** and **2** were synthesized in five and four steps, respectively, starting from 4-nitrophenethyl bromide.[6] Both **1a** and **2** were coupled (via the *N*-hydroxysuccinimide ester) to the carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). Balb/c mice were immunized with the KLH conjugate of **1a** and **2**, and antibodies were generated by standard protocols.[7] Immunization of **1a** produced 23 antibodies while hapten **2** yielded 21 hybridomas. All monoclonals were of the IgG class and were purified from ascites fluid by anion-exchange chromatography followed by affinity chromatography on a protein G column. Antibodies were judged to be homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.[8]

Antibodies at a concentration of 20 μM were initially screened (phosphate buffer 50 mM, pH 7.5, 100 mM NaCl, 37 °C) against benzoate ester **3** (500 μM) for the production of 5-[(4-hydroxyphenyl)amino]-5-oxopentanoic acid (**4**).[9] From the 23 monoclonal antibodies obtained to **1a**, *seven* were found to be catalytic; while *none* of the antibodies to hapten **2** showed any tendency to accelerate the rate of hydrolysis of ester **3**. The seven antibodies that were found to be catalytic were completely inhibited by the addition of free hapten **1b**. Such results suggest that catalysis follows binding of the substrate in the antibody binding pocket. Most significant was the overwhelming number of catalytic antibodies to hapten **1a** vs **2**. One of these seven catalytic antibodies was characterized in detail.

(1) (a) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6736. (b) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science (Washington, D.C.)* **1986**, *234*, 1566. (c) Jacobs, J.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174. (d) Napper, A.; Benkovic, S. J.; Tramontano, A.; Lerner, R. A. *Science (Washington, D.C.)* **1987**, *237*, 1041. (e) Janda, K. D.; Lerner, R. A.; Tramontano, A. *J. Am. Chem. Soc.* **1988**, *110*, 4835. (f) Janda, K. D.; Schloeder, D.; Benkovic, S. J.; Lerner, R. A. *Science (Washington, D.C.)* **1988**, *241*, 1199. (g) Janda, K. D.; Benkovic, S. J.; Lerner, R. A. *Science (Washington, D.C.)* **1989**, *244*, 437.

(2) Lerner, R. A.; Benkovic, S. J. *Bioassays* **1988**, *9*, 107. In this terminology, the hapten serves as "bait" for attracting catalytic functions in the induction of the antibody; it is then "switched" for the substrate.

(3) (a) Pollack, S. J.; Nakayama, G. R.; Schultz, P. G. *Science (Washington, D.C.)* **1988**, *242*, 1038. (b) Pollack, S. J.; Schultz, P. G. *J. Am. Chem. Soc.* **1989**, *111*, 1929.

(4) Baldwin, E.; Schultz, P. G. *Science (Washington, D.C.)* **1989**, *245*, 1104.

(5) (a) Pressman, D.; Grossberg, A. L.; Pence, L. H.; Pauling, L. *J. Am. Chem. Soc.* **1946**, *68*, 250. (b) Pressman, D.; Siegal, M. *J. Am. Chem .Soc.* **1953**, *75*, 686. (c) Grossberg, A. L.; Pressman, D. *J. Am. Chem. Soc.* **1960**, *82*, 5470. (d) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature (London)* **1989**, *338*, 269.

(6) All new compounds exhibited satisfactory spectroscopic (NMR, IR) and combustion analyses (±0.3%).

(7) (a) Kohler, G.; Milstein, C. *Nature (London)* **1975**, *256*, 495. (b) Enguall, E. *Methods Enzymol.* **1980**, *70*, 419.

(8) Laemmli, V. *Nature (London)* **1970**, *227*, 680.

(9) The analysis was performed via HPLC on an RP-C18 column eluting with water–acetonitrile (90:10) at a flow rate of 1 mL/min with UV detector set to 254 nm. The hydrolysis product, **4** (retention time 7 min), was collected and found to be identical by RP-HPLC coinjection and mass spectral analysis with an authentic sample.

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