Steric and Electronic Effects on an Antibody-Catalyzed *Diels–Alder* Reaction

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Dedicated to *Dieter Seebach* on the occasion of his 65th birthday

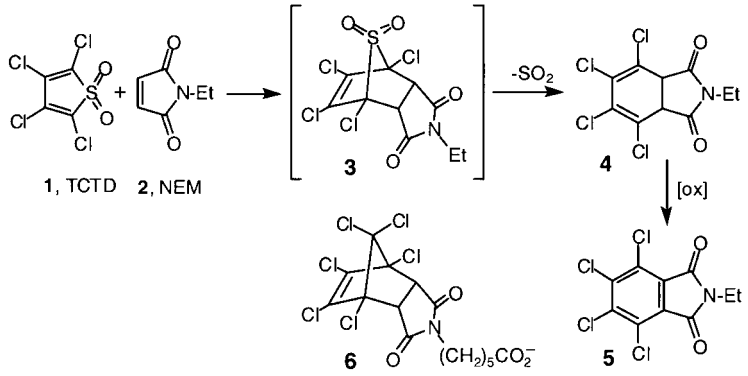
A series of substituted thiophene dioxides was tested as diene substrates for the antibody 1E9, which was elicited with a hexachloronorbornene derivative and normally catalyzes the inverse electron-demand *Diels–Alder* reaction between 2,3,4,5-tetrachlorothiophene dioxide (TCTD) and *N*-ethylmaleimide (NEM). Previous structural and computational studies had suggested that the catalytic efficiency of this system derives in part from a very snug fit between the apolar active site and the transition state of this reaction. Nevertheless, replacing all the Cl-atoms in the hapten with Br-atoms leads to no loss in affinity ($K_d = 0.1$ nM), indicating substantial conformational flexibility in the residues that line the binding pocket. Consistent with this observation, the 2,3,4,5-tetrabromothiophene dioxide is a good substrate for the antibody ($k_{cat} = 1.8\ min^{-1}$, $K_{NEM} = 14$ μM), despite being considerably larger than TCTD. In contrast, normal electron-demand *Diels–Alder* reactions between NEM and unsubstituted thiophene dioxide or 2,3,4,5-tetramethylthiophene dioxide, which are much smaller or nearly isosteric with TCTD, respectively, are not detectably accelerated. These results show that the electronic properties of the 1E9 active site are optimized to a remarkable degree for the inverse electron-demand *Diels–Alder* reaction for which it was designed. Indeed, they appear to play a more important role in catalysis than simple proximity effects.

1. Introduction. – Tailored antibody catalysts are valuable tools for investigating the factors that contribute to the efficiency of biological catalysis [1]. For example, mechanistic and structural investigations of antibodies that promote *Diels–Alder* cycloadditions [2–7] provide insight into how proximity and electrostatic effects can be exploited to increase reaction rate, control *exo/endo* selectivity, and specify absolute configuration in a class of reactions for which natural enzymes have only recently been discovered [8].

Antibody 1E9, for instance, catalyzes the inverse electron-demand *Diels–Alder* reaction between 2,3,4,5-tetrachlorothiophene dioxide (TCTD, **1**) and *N*-ethylmaleimide (NEM, **2**) [2]. The initially formed adduct **3** is unstable and spontaneously eliminates SO_2 to give compound **4** (*Scheme*). Subsequent oxidation *in situ* affords *N*-ethylphthalimide (**5**) as the final product. The antibody is relatively efficient, achieving effective molarities (k_{cat}/k_{uncat}) in excess of 1000M [6]. For comparison, the effective molarities reported for other *Diels–Alderase* antibodies [3] and ribozymes selected directly for their ability to promote cycloadditions [9] rarely exceed 10M.

The hexachloronorbornene derivative **6**, which is an excellent mimic of the transition state for cycloaddition [6], was used as the hapten to generate 1E9. The crystal structure of its complex with the antibody [6] reveals almost perfect shape complementarity between the ligand and the active site, where extensive *Van der Waals* contacts, π-stacking interactions, and a strategically placed H-bond donor are exploited

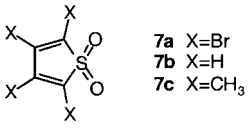
Scheme



for molecular recognition. Computational analysis [7] suggests that the structurally analogous rate-limiting transition state is stabilized enthalpically by its unusually close fit with the apolar binding site. The strong H-bond provided by Asn^{H35} to the dienophile component of the transition state further enhances the catalytic efficacy of 1E9 [7].

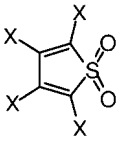
In the experiments outlined below, we have varied the substituents in diene **1** to explore the sensitivity of catalysis by 1E9 to steric and electronic perturbation. We expected to see a close correlation between rate acceleration and the size of the diene. Surprisingly, despite tight packing of the hexachloronorbornene transition-state analog, the 1E9 active site is relatively insensitive to substantial increases in the volume of the substrate. Instead, electronic factors appear to be paramount.

2. Results. – Replacing the Cl-atoms in TCTD (**1**) with Br- and H-atoms, or Me groups (compounds **7a**–**7c**) alters the steric and electronic properties of the diene substantially (*Table*). For example, although **1** and 2,3,4,5-tetrabromothiophene dioxide (**7a**) have similar dipole moments, the latter has a considerably larger steric volume (24.2 Å^3) due to the longer C–Br bond and the larger *Van der Waals* radius of the Br- compared to Cl-atom. Compound **7a** is also substantially more hydrophobic than **1**, as judged by its $c\cdot\log P$ value (1.56 *vs.* 0.897). In contrast, unsubstituted thiophene dioxide (**7b**) is much smaller (by 59.9 Å^3) and less hydrophobic than **1**. 2,3,4,5-Tetramethylthiophene dioxide (**7c**) is comparable in size to **1**, but it is electron-rich and even more hydrophobic than the Br compound.



TCTD (**1**) and thiophene dioxides **7a**–**7c** were prepared according to literature procedures and used as dienes in *Diels–Alder* reactions with *N*-ethylmaleimide (**2**). The uncatalyzed reactions were performed at 25° in 50 mM acetate buffer (pH 5.5) containing 20 mM NaCl and 2% MeCN. With the exception of **7b**, which decomposes

Table. *Calculated*[a]) Van der Waals *Volume, Dipole Moment, and Octanal/H_2O Partitioning Coefficient of Substituted Thiophene Dioxides and Experimental Kinetic Parameters*[b]) *for the* Diels–Alder *Reaction of these Dienes with* N-*Ethylmaleimide* (**2**) *in the Presence and Absence of Antibody*



	Volume [Å^3]	Moment [D]	$c \cdot \log P$	k_{uncat} [M^{-1} min^{-1}]	k_{cat} [min^{-1}]	K_{NEM} [mM]
X = Cl (**1**)	146.9	4.600	0.897	0.040[c])	1.2	7.6
X = Br (**7a**)	171.1	4.977	1.56	0.063	1.8	14
X = H (**7b**)	87.0	6.049	− 0.408	−[d])	–	–
X = Me (**7c**)	153.6	6.902	1.95	0.212	–	–

[a]) The molecular properties of compounds **1** and **7a**–**7c** were calculated by means of the Molecular Analysis Pro program (*Norgwyn Montgomery Software Inc.*, version 3.2). Octanal/H_2O partitioning coefficients were calculated by means of the ACD Labs/logP (version 3.5). [b]) Assays were performed at 25° in 50 mM acetate buffer (pH 5.5) containing 20 mM NaCl and 2% MeCN; [NEM] = 2.5–30 mM, [diene] = 37 μM, [Fab] = 0.36 μM. Standard errors on the kinetic parameters are 15%. For **7b** and **7c**, no reaction over background was observed in the presence of the antibody. [c]) From [2]. [d]) Too unstable in aqueous buffer to be measured.

rapidly in aqueous buffer, formation of the expected cycloaddition products was confirmed by HPLC through comparison with authentic samples; in the case of **7c**, the initially formed dihydrophthalimide subsequently reacts rapidly with a second equiv. of the dienophile to give a bicyclooctene derivative. The kinetics of SO_2 release were determined by monitoring the bleaching of starch-iodine solutions spectroscopically. Values of k_{obs} were determined by the method of initial rates under pseudo-first-order conditions where [NEM] ≫ [diene]; plotting k_{obs} as a function of diene concentration afforded the second-order rate constants k_{uncat} (*Fig. 1*). The reactivity of 2,3,4,5-

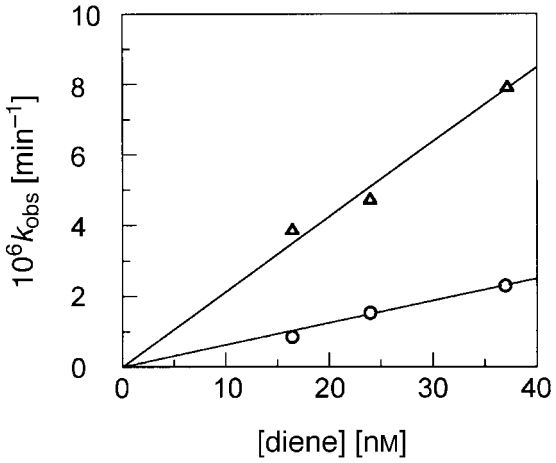


Fig. 1. *Plot of pseudo-first-order rate constants for the reaction of* N-*ethylmaleimide* (**2**) *with 2,3,4,5-tetrabromothiophene dioxide* (**7a**, ○) *and 2,3,4,5-tetramethylthiophene dioxide* (**7c**, △). The k_{obs} values were obtained by the method of initial rates in at 50 mM acetate buffer containing 20 mM NaCl (pH 5.5, 25°).

tetrabromothiophene dioxide (**7a**) is similar to that of TCTD (**1**), whereas the electron-rich 2,3,4,5-tetramethylthiophene dioxide (**7c**) reacts roughly five-times faster (*Table*).

Reactions in the presence of antibody were performed similarly, holding the concentration of the diene constant at 37 μM to minimize problems with solubility. This concentration is far below the K_m value for each diene. Under these conditions, the 1E9-catalyzed cycloaddition between TCTD (**1**) and NEM (**2**) gave an apparent catalytic rate constant, k_{cat}, of 1.2 min^{-1} and a *Michaelis* constant for **2**, K_{NEM}, of 7.6 mM (*Fig. 2*). These values can be compared with the previously published values of 4.5 min^{-1} and 10 mM, respectively, which were obtained at a 4-fold higher diene concentration [10]. As expected, the apparent k_{cat} value is linearly dependent on diene concentration in this concentration range. The 2,3,4,5-tetrabromothiophene dioxide (**7a**) turned out to be a surprisingly good substrate for the antibody, despite its considerably larger steric volume (*Fig. 2* and *Table*), giving apparent k_{cat} and K_{NEM} values 1.5- and 2-times larger than those for **1**, respectively. In contrast, the reactions with thiophene dioxide itself (**7b**) and the electron-rich diene **7c** were not detectably accelerated over background by the antibody, even at high catalyst concentrations (up to 15 μM).

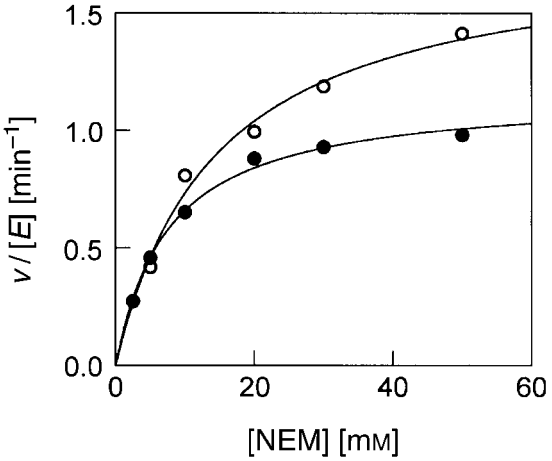


Fig. 2. Michaelis–Menten *plot for the 1E9-catalyzed reaction of* N-*ethylmaleimide* (**2**) *with 2,3,4,5-tetrachlorothiophene dioxide* (**1**, ●) *and 2,3,4,5-tetrabromothiophene dioxide* (**7a**, ○). The assay conditions were the same as those described for the uncatalyzed reactions in *Fig. 1*. The catalyst was the previously described recombinant Fab fragment of antibody 1E9 [10].

In light of the unexpected ability of 1E9 to accept 2,3,4,5-tetrabromothiophene dioxide (**7a**), which is larger than **1**, as a substrate, but not unsubstituted thiophene dioxide **7b**, which is smaller, we synthesized the corresponding hapten analogs **8a** and **8b** for binding studies. These compounds were prepared by the *Diels–Alder* reaction of 6-maleimidocaproic acid and hexabromopentadiene or cyclopentadiene, respectively. Ligand binding to the antibodies was determined by fluorescence titration (*Fig. 3*). Experiments with the original hapten **6** yielded a dissociation constant of 0.10 ± 0.03 nM, in good agreement with the literature [10]. The hexabromonorbornene derivative **8a** is a remarkably good ligand for 1E9, binding as tightly as **6** (K_d = 0.08 ±

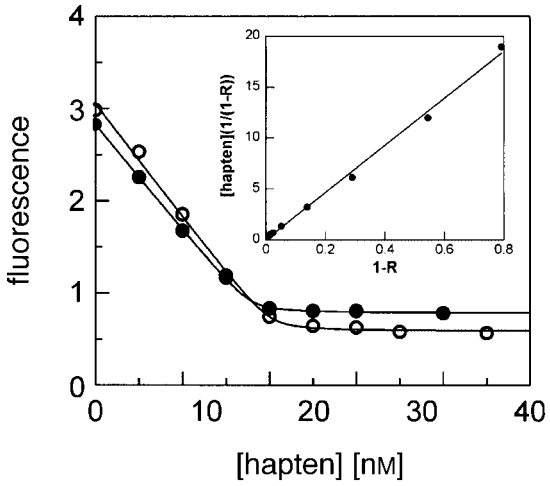
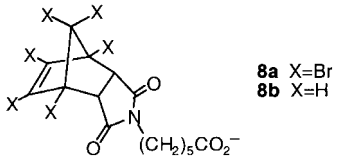


Fig. 3. *Representative fluorescence-titration curves for the binding of hapten* **6** (●) *and the hexabromonorbornene derivative* **8a** (○) *by the 1E9 Fab antibody* [10]. The inset shows a typical *Scatchard* plot for **8a**.

0.1 nM) within experimental error. The unsubstituted norbornene derivative **8b** also binds to the antibody but with an affinity two orders of magnitude lower than that of **6** or **8a** ($K_d = 22 \pm 4$ nM, data not shown).



8a X=Br
8b X=H

3. Discussion. – The complex between antibody 1E9 and hapten **6** is characterized by an unusually snug fit [6][7]. Approximately 84% of the ligand surface is buried upon binding, and the gap volume, defined as the difference between the volume of the complex and the sum of the volumes of the antibody and the hapten, is only 36.8 Å^3. The shape complementarity to the hexachloronorbornene portion of the ligand is particularly good.

Nevertheless, 1E9, like related antibodies [4][11], also cross-reacts with unrelated hydrophobic ligands, albeit with reduced affinity [5]. Computational docking experiments [6] have suggested that noncognate ligands bind more or less randomly in the apolar cavity, whereas the original, high affinity hapten adopts a highly specific binding mode corresponding to that seen in the crystal structure. The ability of 1E9 to recognize the unsubstituted norbornene derivative **8b** is consequently unsurprising. This compound, which has a *Van der Waals* volume of 284.9 Å^3, is considerably smaller than **6** (366.8 Å^3) and should fit well within the pocket. Its 200-fold lower affinity can presumably be attributed to less optimal shape complementarity with the binding site.

Unexpectedly, however, replacing all the Cl-atoms in the hapten with Br-atoms causes no change in hapten affinity. Even though the resulting hexabromonorbornene

derivative has a substantially larger *Van der Waals* volume (418.3 Å^3) than the original hapten (366.8 Å^3) and cannot be sterically accommodated within the active site without substantial adjustment of the backbone and side chains lining the cavity, compounds **6** and **8a** are bound by 1E9 equally tightly. The high affinity of this interaction ($K_d \approx$ 0.1 nM) is most consistent with a single binding mode similar to that of **6** and suggests that the 1E9 binding pocket possesses an inherent flexibility that allows it to expand somewhat to match the steric requirements of the larger ligand. Steroid binding to the related antibody DB3 is known to be accompanied by conformational changes that produce a dramatic alteration in the shape of the binding site [12].

The observation that tetrabromothiophene dioxide is a good substrate for the antibody argues in favor of this hypothesis. The ratio k_{cat}/k_{uncat} is essentially identical to that for **1** at subsaturating diene concentrations, and because the *Michaelis* constant for NEM, K_{NEM}, is slightly elevated, the apparent second-order rate constant for the reaction, k_{cat}/K_{NEM}, is only two-fold smaller than for **1**. These results are consistent with effective ordering of the reactants in the transition state at the active site. Insofar as K_{NEM} reflects substrate affinity, dienophile binding becomes more difficult in the presence of the more voluminous diene as might be expected. However, once both reactants are bound, the *Diels–Alder* reaction can occur efficiently. The tighter fit of the larger tetrabromothiophene dioxide (**7a**) may actually facilitate the bond-forming step by reducing the degrees of freedom in the system needed to constrain the substrates in a reactive geometry.

Clearly, size is not the only factor at play in this system. The electron-rich diene, tetramethylthiophene dioxide (**7c**), is not a substrate for the antibody, even though it is only marginally larger than **1** (*Table*). Despite its greater hydrophobicity, which should favor partitioning into the apolar active site, and its intrinsically higher reactivity, no detectable catalysis over background was observed. The lack of activity of the unsubstituted thiophene dioxide (**7b**) is less surprising, given its instability. The small size of this diene would also allow it to bind in multiple, generally unproductive orientations within the binding pocket. The fact that 1E9 catalyzes the inverse electron-demand *Diels–Alder* reaction of **1** and **7a** with NEM but not the normal electron-demand reaction of **7b** and **7c** strongly suggests that electronic factors are much more important to catalytic efficiency in this system than simple shape complementarity.

Our results indicate that the electronic properties of the 1E9 active site are optimized to a remarkable degree for the inverse electron-demand *Diels–Alder* reaction for which it was designed. In addition, the ability of the pocket to accommodate electron-deficient dienes like 2,3,4,5-tetrabromothiophene dioxide (**7a**), which are substantially larger than TCTD (**1**), provides evidence for considerable dynamic flexibility of the binding pocket. Modifying the electronic and dynamic properties of the binding pocket through mutagenesis may provide insight into the molecular origins of the effects observed in this system as well as the means for further augmenting catalytic efficacy.

Experimental Part

General. Reagent-grade solvents and reagents were purchased from commercial suppliers and used without further purification. Literature procedures were used to prepare *2,3,4,5-tetrabromothiophene dioxide* (**7a**) [13], *thiophene dioxide* (**7b**) [14], and *2,3,4,5-tetramethylthiophene dioxide* (**7c**) [15]. Cyclopentadiene was obtained by cracking dicyclopentadiene over paraffin oil. Hexabromocyclopentadiene was synthesized by a published protocol [16]. The full-length 1E9 IgG [17] and its recombinant Fab fragment [10] were prepared and purified as described previously. Reversed-phase HPLC: *Waters* HPLC system equipped with a diode array detector (*UV600LP*, *Thermo Separation Products*) with an *M&N Nucleosil C-18 100-5* column (4.6 × 250 mm); the identity of eluted compounds was verified by comparison with authentic samples. M.p.: uncorrected. ^{1}H- and ^{13}C-NMR Spectra: *Varian* 300-, 400-, and 500-MHz instruments at 298 K; chemical shifts (δ) in ppm are referenced to Me_4Si. MALDI-MS: *IonSpec Ultima FTMS* mass spectrometer. EI-MS: *VG Tribrid* instrument (*Macromass*). Kinetic measurements were preformed on a *Perkin-Elmer* model *Lambda 40* spectrometer. Fluorescence titrations were measured on a *AMINCO●Bowman Series 2* luminescence spectrometer.

6-(1,7,8,9,10,10-Hexabromo-3,5-dioxo-4-azatricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)hexanoic Acid (**8a**). A mixture of hexabromocyclopentadiene (1.25 g, 2.36 mmol) and 6-maleimidocaproic acid (0.5 g, 2.36 mmol) in toluene (10 ml) was heated under reflux while stirring for 24 h. The solvent was evaporated, and the crude brown solid was dissolved in 10% Na_2CO_3 soln. (60 ml). The soln. was washed twice with Et_2O. The aq. layer was acidified to pH 1–2 with HCl and extracted three times with Et_2O. The combined Et_2O extracts were washed with H_2O and dried (Na_2SO_4). After evaporation of the solvent, the crude product (R_f 18 min) was purified by RP-HPLC with an MeCN (0.05% TFA)/H_2O (0.1% TFA) gradient, starting at 40% MeCN and increasing to 90% MeCN over 25 min, at an elution rate of 10 ml/min. Compound **8a** was obtained after lyophilization as a white powder (1.2 g, 65%). M.p. = 66–68°. ^{1}H-NMR (500 MHz, CD_3CN): 4.01 (*s*, 2 H); 3.33 (*t*, 2 H); 2.26 (*t*, 2 H); 1.56 (*m*, 2 H); 1.46 (*m*, 2 H); 1.31 (*m*, 2 H). ^{13}C-NMR (125 MHz, CD_3CN): 174.93; 171.53; 128.01; 86.40; 73.74; 39.95; 33.94; 29.65; 28.38; 27.02; 24.16. HR-MALDI-MS: 751.5950 ($C_{15}H_{13}Br_6NO_4$, $[M+H]^+$; calc. 751.5957).

6-(3,5-Dioxo-4-azatricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)hexanoic Acid (**8b**). Cyclopentadiene (0.17 g, 2.50 mmol) was added to a soln. of 6-maleimidocaproic acid (0.5 g, 2.36 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred at 25° for 2 h. The solvent was evaporated, and the crude product was dried under high vacuum for 8 h. The crude yellow oil was dissolved in 10% Na_2CO_3 (50 ml) and washed twice with Et_2O. The aq. layer was acidified to pH 1–2 with HCl and extracted three times with Et_2O. The combined Et_2O extracts were washed with H_2O and dried (Na_2SO_4). The product (R_f 10 min) was purified by RP-HPLC with an MeCN (0.05% TFA)/H_2O (0.1% TFA) gradient, starting at 30% MeCN and increasing to 90% MeCN over 25 min, at an elution rate of 10 ml/min. Compound **8b** was obtained as a colorless oil (0.4 g, 74%). ^{1}H-NMR (300 MHz, CD_3CN): 6.04 (*s*, 2 H); 3.23 (*m*, 4 H); 2.24 (*t*, 2 H); 1.63 (*t*, 1 H); 1.60 (*t*, 1 H); 1.51 (*m*, 4 H); 1.38 (*m*, 2 H); 1.23 (*m*, 2 H). ^{13}C-NMR (75 MHz, CD_3CN): 178.44; 175.32; 135.08; 52.62; 46.34; 45.50; 38.52; 33.88; 28.10; 26.87; 24.97. HR-MALDI-MS: 300.1202 ($C_{15}H_{19}NO_4$, $[M+Na]^+$; calc. 300.1212).

*4,5,6,7-Tetrabromo-2-ethyl-3a,7a-dihydro-2*H*-isoindole-1,3-dione.* A mixture of **7a** (48.3 mg, 0.11 mmol) and N-*ethylmaleimide* (**2**; 14.2 mg, 0.11 mmol) in 1,2-dichloroethane (1.8 ml) was heated under reflux while stirring for 1 h. The solvent was evaporated, and the residue was purified by column chromatography (SiO_2; hexane/AcOEt 6:1). The product was obtained as a pale yellow powder (32 mg, 60%). TLC (hexane/AcOEt 6:1): R_f 0.42. M.p. 177–178°. ^{1}H-NMR (300 MHz, CD_3CN): 4.16 (*s*, 2 H); 3.49 (*q*, 2 H); 1.12 (*t*, 3 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.25; 119.96; 117.72; 50.90; 35.31; 12.81. HR-EI-MS: 494.7147 ($C_{10}H_7Br_4NO_2$, M^+; calc. 494.7144).

4,10-Diethyl-1,7,13,14-tetramethyl-4,10-diazatetracyclo[5.5.2.0^{2,6}.0^{8,12}]tetradec-13-ene-3,5,9,11-tetraone. A mixture of **7c** (12.5 mg, 0.07 mmol) and **2** (11.4 mg, 0.09 mmol) in 1,2-dichloroethane (1.0 ml) was heated under reflux while stirring for 1 h. The solvent was evaporated, and the residue was purified by column chromatography (SiO_2; hexane/AcOEt 2:1) to give the title compound as a white powder (7.9 mg, 49%). TLC (hexane/AcOEt 1:1): R_f 0.42. M.p. 79–80°. ^{1}H-NMR (400 MHz, CD_3CN):3.34 (*q*, 2 H); 2.62 (*s*, 2 H); 1.82 (*s*, 3 H); 1.50 (*s*, 3 H); 0.90 (*t*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 175.73; 131.59; 48.68; 34.21; 32.40; 29.05; 22.09; 18.77. HR-MALDI-MS: 359.1964 ($C_{20}H_{27}N_2O_4$, $[M+H]^+$; calc. 359.1965).

Kinetic Assays. Reaction between the different thiophene dioxides, **1** and **7a**–**7c**, and N-*ethylmaleimide* (**2**; NEM) was measured in 50 mM acetate, 20 mM NaCl, pH 5.5 at 25° in the presence and absence of antibody. The substrates were diluted from stock solns. in MeCN; the final concentration of MeCN in the reaction mixture was 2% by volume. Initial velocities were determined by starch-I_2 bleaching at 606 nm [5][6] at several concentrations of **2** while holding the concentration of diene constant and at least ten-times lower than that of the dienophile. Second-order rate constants for the uncatalyzed reactions were then obtained by plotting the

apparent first-order rate constants as a function of diene concentration. Initial rates for the antibody-catalyzed reactions were corrected for the uncatalyzed reaction and fitted to the *Michaelis–Menten* equation, $v_o/[Ab] = k_{cat}[NEM]/(K_{NEM} + [NEM])$, where v_o is the initial rate, [Ab] and [NEM] are the active site and substrate concentrations, respectively, and k_{cat} and K_{NEM} are the catalytic rate and the *Michaelis* constants at a fixed concentration of diene, respectively. Concentrations of **2** were varied from 2.5 to 30 mM, and the concentration of the different thiophene dioxides was held constant at 37 μM. Both IgG [17] and the recombinant Fab fragment of 1E9 [10] were used at binding-site concentrations ranging from *ca.* 1 μM to 15 μM.

The reaction of **7c** with **2** in the presence of high concencentrations of antibody was also monitored at 305 nm by RP-HPLC. The disappearance of **7c** was quantified with acetophenone as internal standard. For these experiments, 1E9 IgG was methylated by the method of *Jenthoft* and *Dearborn* [18], and dialyzed into the reaction buffer prior to use.

Fluorescence Titration. The affinity of norbornenes **6**, **8a**, and **8b** for antibody 1E9 was determined by fluorescence titration in 50 mM acetate buffer, 20 mM NaCl, pH 5.5 and at 15° [19]. Titration curves were recorded by stepwise addition of a stock soln. of ligand (15 μM) to a diluted soln. of the 1E9 Fab fragment (26 nM) and subsequent measurement of the fluorescence. Tryptophan emission was monitored at 340 nm following excitation at 290 nm. Fluorescence intensity was recorded in the absence of ligand (F_E), with excess ligand (F_{EL}), and as a function of increasing ligand concentration at a fixed concentration of antibody (F). All emission spectra were corrected by subtracting appropriate background spectra. Dissociation constants (K_d) were obtained by *Scatchard* analysis with the equation $(L_T)(1/R-1) = K_d + nE_T(1-R)$, where L_T is the total ligand concentration, n is the number of ligand binding sites, E_T is the total antibody concentration, and R is the ratio of the antibody–ligand complex to total antibody concentration (E_L/E_T). The ratio R was quantified by measuring $(F_E - F)/(F_E - F_{EL})$ at a given ligand concentration L_T.

Calculations. The *Van der Waals* volume and dipole moment of substituted thiophene dioxides and norbornene derivatives were calculated by means of the Molecular Analysis Pro program (*Norgwyn Montgomery Software Inc.*, version 3.2). Octanal/H_2O partitioning coefficients were calculated by means of the ACD Labs/logP (version 3.5).

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