# First Example of an Antibody-catalysed Hetero-Diels–Alder Reaction

**Arthur A. P. Meekel, Marina Resmini and Upendra K. Pandit***

*Organic Chemistry Laboratory, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands*

Two transition-state directed antibodies catalyse a Diels–Alder reaction between a 1,3-diene and an arylnitroso dienophile; one of the antibodies shows a good rate enhancement ($k_{cat}/k_{uncat}$ = 1205) and steers the reaction in favour of the targeted isomeric adduct.



The Diels–Alder reaction is a versatile tool in organic chemistry for the synthesis of six-membered ring compounds. The reaction between a conjugated diene and a dienophile is a bimolecular process characterised by a large entropic barrier, with $\Delta S^{\ddagger}$ values in the range of −30 to −40 cal $K^{-1}$ $mol^{-1}$ (1 cal = 4.184 J).[1] No natural enzymes are known which affect the course of these cycloaddition reactions; this, together with recent studies on the generation of tailored catalysts from the immune system,[2] suggests that antibodies, raised against stable transition-state analogues, could be used to accelerate the rate of the reaction. Several groups have successfully isolated specific antibodies with catalytic activity for the Diels–Alder reaction.[3] Whereas these studies have dealt with the formation of carbocyclic systems, we report the first example of an antibody-catalysed hetero-Diels–Alder reaction.

The reaction selected for investigation was the addition of the unsymmetrical diene (*trans*-piperylene) **1** to the nitroso dienophile **2** (Scheme 1). This choice was influenced by the following considerations: (i) the cycloaddition of nitroso derivatives to dienes is a synthetically and mechanistically interesting reaction;[4] (ii) the two heteroatoms in the dienophile eliminate the potential formation of *exo*- and *endo*-isomeric products and allow only the possible generation of the two regioisomers **3** and **4** as racemates; (iii) the methyl group in the diene gives the opportunity to test whether the antibodies raised against the isomeric haptens **5** and **6** can steer the course of the reaction towards the formation of the targeted regioisomer; furthermore, the substituent allows an analysis of the enantiomeric selectivity of the antibody-catalysed reaction. By choosing the methyl group we wanted to investigate the effects of the smallest carbon-bearing substituent.

Haptens **5** and **6** were synthesised by the reaction of 1-methylcyclohexa-1,3-diene with methyl 4-nitrosobenzoate, followed by separation of the two regioisomeric adducts and subsequent transformation of the ester group into the spacer required for coupling with immunogenic proteins. The conjugates with cationised bovine serum albumin and chicken γ-globulins were used to immunise Balb/c mice, following the standard protocol. The selected antibodies were screened for catalytic activity and the two antibodies, namely 290-4B10 against hapten **5** and 309-1G7 against hapten **6**, that showed significant rate enhancement, were amplified for further characterisation.†

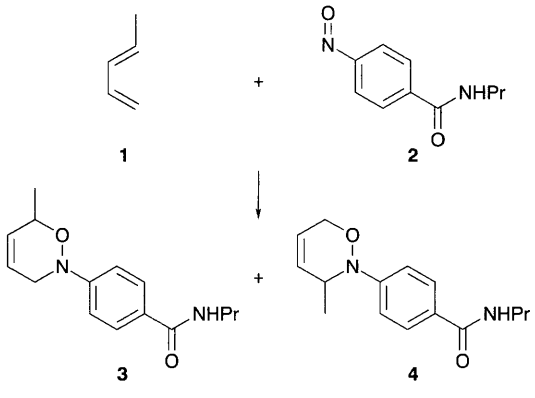
The rate of the reaction between **1** and **2** was measured under pseudo first order conditions‡ by monitoring the disappearance of the nitroso dienophile at 340 nm, a wavelength at which there is no interfering absorption by the diene or the products of the reaction. In the presence of the antibodies, initial rates were measured within 5% completion and corrected for the background reaction. The isomeric distribution of the products was determined by analytical reversed phase HPLC.§

The pseudo first order rate constant for the background reaction was found to be 3.1 ± 0.3 × $10^{-4}$ $s^{-1}$.¶ Reactions carried out in the presence of antibodies 290-4B10 and 309-1G7 followed Michaelis Menten kinetics; Lineweaver Burk plots were constructed that gave the following data for 290-4B10 (14.6 μmol $dm^{-3}$): $K_m$ = 2.94 mmol $dm^{-3}$ for dienophile **2**, $V_{max(app)}$ = 1.38 × $10^{-3}$ mmol $dm^{-3}$ $s^{-1}$, $k_{cat(app)}$ = 9.41 × $10^{-2}$ $s^{-1}$. This resulted in a $k_{cat}/k_{uncat}$ value of 277. For 309-1G7 (9.4 μmol $dm^{-3}$) the results were highly significant, namely $K_m$ = 3.11 mmol $dm^{-3}$ for dienophile **2**, $V_{max(app)}$ = 3.17 × $10^{-3}$ mmol $dm^{-3}$ $s^{-1}$, $k_{cat(app)}$ = 3.37 × $10^{-1}$ $s^{-1}$, from which a $k_{cat}/k_{uncat}$ value of 1205 was obtained.

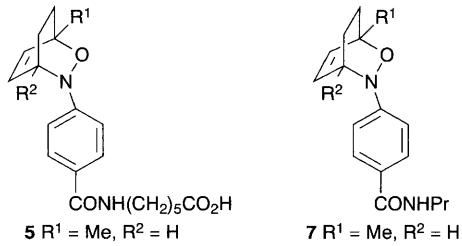
Addition of the corresponding inhibitors, *i.e.* **7** for 290-4B10 and **8** for 309-1G7, to the antibody-catalysed reactions gave complete inhibition, with the rate dropping to the background value, thus revealing that catalysis is taking place in the antibody binding sites. Under the same conditions, controls were performed by using non-specific protein and normal mouse immunoglobulin G, which showed no influence on the reaction rate.

The kinetic data show that the two antibodies catalyse the selected hetero-Diels–Alder reaction, albeit with different efficiencies. It follows from these results that, as shown for carbocyclic systems, the hetero-cycloaddition studied has a product-like transition state that can be suitably mimicked by bicyclic adducts like **5** and **6**. The antibody-binding site presumably prealigns the diene and the dienophile and thereby minimises the loss of rotational and translational entropy.

Table 1 reveals that antibody 290-4B10, raised against hapten **5**, causes no observable change in the ratio of the two isomeric products, compared to the background value. On the other hand, antibody 309-1G7, from hapten **6**, causes a distinct shift of this ratio in the direction governed by the hapten structure. In this context, it should be noted that the ratios expressed in Table 1 are derived directly from the total products formed, *i.e.* they have not been corrected for the background reaction. The difference in the ratio of the regioisomers became more distinct




**Scheme 1** Reaction between **1** and **2** carried out in PBS–5% $Me_2SO$ at room temp.; yields: **3**, 58%; **4**, 42%




**5** $R^1$ = Me, $R^2$ = H
**6** $R^1$ = H, $R^2$ = Me
**7** $R^1$ = Me, $R^2$ = H
**8** $R^1$ = H, $R^2$ = Me

**Table 1** The influence of the antibodies on the distribution of the regioisomers **3** and **4**

| Antibody | [Ab]/μmol dm$^{-3}$ | [**2**]/μmol dm$^{-3}$ | **3**:**4** |
|---|---|---|---|
| none | | [a] | 58:42 |
| 290-4B10 | 14.6 | 146 | 58:42 |
| 290-4B10 | 36.6 | 36.6 | 58:42 |
| 309-1G7 | 9.4 | 94 | 51:49 |
| 309-1G7 | 33.3 | 33.3 | 47:53 |

[a] At all concentrations of **2** examined, the same product distribution was observed.

when the reaction was carried out with 1 equiv. of antibody 309-1G7 in order to eliminate the effect of product inhibition. It is obvious that when the catalytic effect of the antibody is low, the product ratio will be dominated by the high rate of the background reaction. This is consistent with the results found for the two antibodies.

An approach to obtain more detailed information on the activity of the antibody, *i.e.* rate enhancement and product distribution, would be to suppress the rate of the background reaction to low values. Efforts in this direction involving modification of the substrate structure are currently in progress.

We wish to express our indebtedness to the late Professor B. F. van Gelder for his interest and helpful discussions. The immunological work was carried out at the E. C. Slater Institute, University of Amsterdam. This work was in part conducted under the auspices of the Stichting Scheikundig Onderzoek Nederland (SON) with the financial support of the Netherlands Organisation of Scientific Research (NWO) and of the European Network on Antibody Catalysis, Human Capital and Mobility Programme (post-doctoral fellowship to M. R.).

*Received, 1st December 1994; Com. 4/07355J*

## Footnotes

† Hybridomas were generated by standard methods using SP2/O$^+$ myeloma cells as the fusion partner, and were subsequently screened by enzyme-linked immunosorbent assay (ELISA) for binding. The selected clones were amplified in stationary 1 l cultures and antibodies were purified by protein G affinity chromatography. Antibody concentrations were determined by measuring the absorbance at 280 nm, using $\varepsilon_{280}$ = 202 500 dm$^3$ cm$^{-1}$ mol$^{-1}$ and a molecular mass of 150 000.

‡ Reactions were carried out in phosphate-buffered saline (PBS) 0.01 mol dm$^{-3}$, pH 7.3 containing 5% $Me_2SO$; the concentration of diene **1** is fixed at 5 mmol dm$^{-3}$, the concentration of the dienophile **2** is varied from 0.13 to 0.67 mmol dm$^{-3}$. Higher concentrations were not applied owing to the poor solubility of the substrate **2**.

§ HPLC measurements were performed using a C18 reversed-phase column, employing isocratic conditions: MeOH : $H_2O$ 4 : 6 containing 0.1% TFA, with UV detection at 282 nm; retention time for **2**, 8.9 min; for **4**, 28 min; and for **3**, 31.5 min.

¶ The rate of the uncatalysed reaction was measured simultaneously with each antibody-mediated reaction, in order to ensure constant experimental conditions.

## References

1 M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 1678.

2 R. A. Lerner, S. J. Benkovic and P. G. Schultz, *Science*, 1991, **252**, 659.

3 D. Hilvert, K. W. Hill, K. D. Nared and M.-T. Auditor, *J. Am. Chem. Soc.*, 1989, **111**, 9261; A. C. Braisted and P. G. Schultz, *J. Am. Chem. Soc.*, 1990, **112**, 7430; C. J. Suckling, M. C. Tetford, L. M. Bence, J. I. Irvine and W. H. Stimson, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1925; V. E. Gouverneur, K. N.Houk, B. de Pascual-Teresa, B. Beno, K. D. Janda and R. A. Lerner, *Science*, 1993, **262**, 204.

4 D. L. Boger and S. N. Weinreb, *Hetero Diels–Alder Methodology in Organic Synthesis*, Academic, London, 1987; G. W. Kirby, *Chem. Soc. Rev.*, 1977, **6**, 1; G. Kresze and J. Firl, *Fortsch. Chem. Forsch.*, 1969, **11**, 245.