

**Antibody-Catalyzed Enantioselective Norrish Type II Cyclization\*\****Sigal Saphier, Subhash C. Sinha, and Ehud Keinan\**

The Norrish type II photochemical reaction involves abstraction of a  $\gamma$  hydrogen atom by an excited carbonyl oxygen atom (e.g. in **1**) to produce a 1,4-diradical intermediate, such as **A**.<sup>[1]</sup> The latter can undergo three possible reactions: a) reverse hydrogen transfer to regenerate the ground state of **1**; b) C–C-bond cleavage to form an alkene **2** and an enol that tautomerizes to the carbonyl compound **3**; or c) radical recombination (Yang cyclization<sup>[2]</sup>) to produce the cyclobutanols **4** and **5** (Scheme 1). Usually, pathway b is the most common route, while c) represents a minor side reaction. Intense mechanistic studies over the past three decades have made the Norrish type II reaction one of the most well-understood photochemical reactions.<sup>[1]</sup> Unfortunately, this important reaction has not yet enjoyed comparable status in synthetic chemistry, mainly as a result of a lack of control over product selectivity and, in particular, stereoselectivity.<sup>[3]</sup>

The most successful approach to limiting the broad variety of possible photoproducts has been based on solid-state reactions, in which the crystal-packing forces severely restrict the range of available conformations.<sup>[4]</sup> However, for syn-

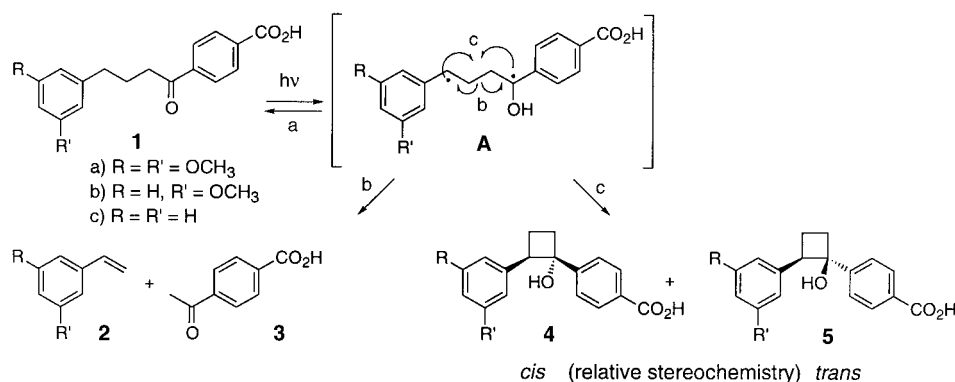
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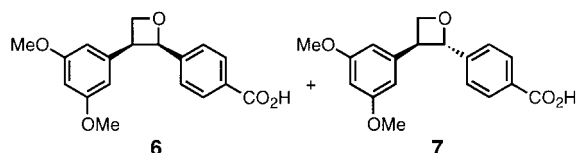
**Scheme 1.** Norrish type II reaction with possible reaction pathways of the 1,4-diradical intermediate.

thetic purposes this approach is limited to cases in which the reactant conformation is photochemically productive. Moreover, it is difficult to predict the preferred solid-state conformation of a given compound. Other attempts to control the reaction by using various organized media that restrict the conformational flexibility of the diradical intermediate have enjoyed limited success.<sup>[5]</sup>

Biocatalysis with enzymes represents an attractive strategy to restrain mechanistic manifolds and channel a reactive intermediate into a single product.<sup>[6]</sup> Unfortunately, the natural diversity of enzymes that catalyze photochemical reactions is rather limited. Aside from the highly complex protein assemblies involved in light-harvesting systems,<sup>[7]</sup> only three enzymes that catalyze a specific photoreaction have been reported.<sup>[8]</sup> These include protochlorophyllide reductase<sup>[9]</sup> and two enzymes that catalyze the fragmentation of thymine dimers in the DNA-repair mechanism: DNA photolyase<sup>[10]</sup> and [6-4]-photoproduct lyase.<sup>[11]</sup> It is clear that evolution has selected against photoenzymes, particularly in multicellular organisms.

The deficiency in natural catalysts calls for the design of new ones, for example, catalytic antibodies. The three reported attempts to catalyze photoreactions with antibodies are the dimerization of methyl *p*-nitrocinamate,<sup>[12]</sup> the cleavage of thymine and uracil cyclobutane dimers,<sup>[13]</sup> and intramolecular hydrogen transfer in an  $\alpha$ -ketoamide.<sup>[14]</sup>

Scheffer reported the successful photocyclization of ketones in the solid state.<sup>[4]</sup> We envisioned that antibodies elicited against the *cis* and *trans* oxetanes **6** and **7** would



**Scheme 2.** Haptens used for immunization.

restrict the conformational flexibility of **1** and stabilize its productive conformation for photochemical cyclization. Herein we report that antibodies that were elicited against **6** and **7** (Scheme 2) do indeed selectively catalyze the formation of a cyclobutanol product in the Norrish type II reaction.

Furthermore, we show that the resultant photoproduct is obtained as a single stereoisomer with very high enantioselectivity.

Each of the isomeric haptens **6** and **7** were synthesized as racemic mixtures and fully characterized (see Supporting Information). They were then mixed in a 1:1 ratio and the mixture was conjugated to KLH (keyhole limpet hemocyanin) and to BSA (bovine serum albumin) using known procedures.<sup>[15a]</sup> The KLH conjugate was used to elicit monoclonal antibodies by standard hybridoma technology.<sup>[15b]</sup> Preliminary ELISA (enzyme-linked immunosorbent assay) screening for the best binders of **6** and **7** using the BSA conjugate<sup>[15c]</sup> afforded 800 antibody-producing hybridoma cell lines. As we were interested in antibodies that bind the entire substrate molecule in the desired conformation, we selected the 60 best binders based on their selective recognition of either **6** or **7**. Assuming that the desired catalysts should also bind the substrate, we carried out further ELISA selection for binders of the **1**-BSA conjugate and thus narrowed the library down to ten antibodies. Our assumption was corroborated later by the fact that all three catalysts discovered for the reaction were in the narrow library of the ten anti-**1** antibodies, while no catalyst was found in the remaining group of 50 antibodies that bind **6** and/or **7** well, but do not bind **1**.

Antibodies were tested for catalysis by irradiation in solution in the presence of **1** (with either a xenon or a mercury UV lamp). Three antibodies, 12B4, 20F10, and 21H9, catalyzed the formation of the *cis*-cyclobutanols **4**, the structure and relative stereochemistry of which were determined by comparison (<sup>1</sup>H NMR, MS, UV and HPLC) with authentic synthetic samples (see Supporting Information). No reaction was observed in the dark, either in the presence or absence of antibodies.

The possibility of carrying out such an enantioselective cyclization is of particular interest because it is generally difficult to achieve stereochemical control over reactions that involve radical intermediates. We used HPLC with a chiral column to measure the enantioselectivity of the antibody-catalyzed photochemical cyclization. The chromatograms were compared with those of authentic racemic samples of **4**, which was synthesized independently (see Supporting Information). Interestingly, all three antibodies produced the

same enantiomer of **4**, the absolute configuration of which is yet unknown (antibody, *ee*): 20F10, 96%; 12B4, 80%; 21H9, 78%; for R = R' = OMe.

The most enantioselective antibody, 20F10, also yielded the highest cyclization/fragmentation ratio (70:30) at 312 nm. Therefore, we selected 20F10 for kinetic studies and further characterization. At low antibody concentrations, the background photochemical cleavage reaction becomes dominant. This precluded measurements of the initial rates for the cyclization under Michaelis–Menten approximation, in which low catalyst concentrations relative to substrate loadings are normally used to determine the kinetic parameters ( $k_{\text{cat}}$  and  $K_{\text{M}}$ ). To circumvent this problem we turned to an alternative model, which uses excess catalyst with constant substrate concentration. Under these conditions, rate constants were obtained from a series of pseudo-first-order kinetic data (see Supporting Information). Data were modeled according to the general format of Michaelis–Menten as described by Klotz and co-workers.<sup>[16]</sup> As expected, the rate constant is highly dependent on the flux of light. For example, when a 150-W Xenon lamp was used the reaction was complete within 10 s. This high  $k_{\text{cat}}$  made it technically difficult to obtain accurate results. We therefore used a weaker, 75-W Xenon lamp to delay the reaction over a time span of 20 min. Under these conditions, we obtained the values of  $k_{\text{cat}} = 0.008 \pm 0.001 \text{ min}^{-1}$  and  $K_{\text{M}} = 58 \pm 13 \text{ }\mu\text{M}$ . Much higher values of  $k_{\text{cat}}$  would be obtained with more intense UV lamps. No cyclization products were detected in the absence of the antibody.

To evaluate the catalytic efficiency of the antibody over multiple turnovers, the antibody was recycled several times by dialysis and its activity was examined. The photochemical reaction was performed in each cycle with equal concentrations of antibody and substrate. No reduction in activity was observed after five cycles, indicating that the active site of the protein is remarkably stable under the photochemical conditions. The stability of antibodies under UV radiation seems to be a general phenomenon.<sup>[17]</sup> Moreover, the ability of the active sites of antibodies to handle reactive intermediates, including free radicals, carbonium ions<sup>[18]</sup> and reactive oxygen species,<sup>[17]</sup> is of particular significance to the general field of biocatalysis. Total product formation amounted to more than three times the initial concentration of the antibody. This observation, together with the stability of the catalyst, suggest that, in principle, the catalyst could be immobilized and used in a flow system for continuous synthesis of the photo-product.<sup>[19]</sup>

The dependence of cyclization efficiency on the irradiation wavelength within the range of 245–320 nm was examined (see Supporting Information). The relative rates followed the antibody absorption spectrum quite faithfully, with a red shift of approximately 10 nm (maximal activity at 290 nm). This suggests the presence of a possible active site tryptophan, which acts as a sensitizer and transfers the light energy to the substrate.<sup>[13a]</sup> By contrast, the fragmentation efficiency was found to follow the absorption spectrum of the substrate ( $\lambda_{\text{max}} = 250 \text{ nm}$ ). The different wavelength dependence of the two reaction routes could be exploited to optimize the cyclization yield. Indeed, the cyclization/fragmentation ratio was increased from 37:63 at 240 nm to 70:30 at 312 nm.

As substrate **1** is inert in the dark, it is possible to measure its binding constants to the antibody directly. Isothermal titration calorimetry (ITC) measurements afforded the binding constants of substrates and haptens (compound,  $K_{\text{a}}$  ( $\text{M}^{-1}$ )): **6**,  $5.89 \times 10^4$ ; **7**,  $2.10 \times 10^5$ ; **1a**,  $1.92 \times 10^6$ ; **1b**,  $1.2 \times 10^4$ ; **1c**,  $< 5 \times 10^3$ . Interestingly, the methoxy groups in the substrate play a crucial role in binding, as reflected by the significant drop in binding upon removal of one or two methoxy groups. The trend in substrate binding was found to parallel the relative efficiency in forming the corresponding *cis* cyclobutanol product in the presence of antibody 20F10 (substrate, relative rate): **1a**, 8; **1b**, 2; **1c**, 1.

Remarkably, 20F10 binds substrate **1a** more strongly than it binds either of the two haptens. Furthermore, 20F10 catalyzes the formation of a *cis*-diaryl cyclobutanol **4**, and yet it binds the *trans* oxetane **7** more tightly than the *cis* isomer **6**. Apparently, **1a** is sufficiently flexible to assume the optimal binding conformation, which seems to be an intermediate structure between **6** and **7**. It has been proposed that the cleavage of 1,4-biradicals requires good overlap between the radical-containing p orbitals and the C2–C3 bond.<sup>[4a]</sup> Failing that, and provided that C1 and C4 are within reasonable bonding distance, cyclization will predominate. Thus, it is conceivable that the protein imposes conformational constraints on the substrate, which enforce preferential cyclization.

In conclusion, the first antibody-catalyzed Yang cyclization has been developed, producing a single stereoisomeric product with very high enantioselectivity. Catalysis by the antibody probably involves the dual effects of stabilizing the productive conformation as well as light harvesting and energy transfer. Further work towards a better understanding of these effects is currently underway.

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