Remarkable Ability of Different Antibody Catalysts To Control and Diversify the Product Outcome of Cationic Cyclization Reactions

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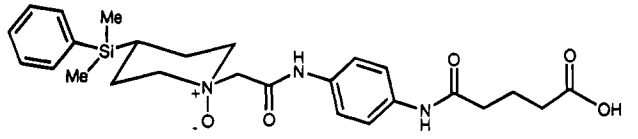
The utilization of transition-state theory in the design of haptens for the production of monoclonal antibodies to catalyze chemical reactions has proven to be quite general.[1] We[2] and others[3] have argued that for many reactions an important design element of the hapten is the strategic distribution of charges. The main idea is to induce functionalities in the antibody capable of interacting with developing charges in the rate-limiting transition state. The essence of this tack, which we have termed "bait and switch" catalysis, involves the placement of a point charge or charges within the hapten in close proximity to, or in direct substitution for, a chemical functional group we wish to transform in the prospective substrate. The haptenic charge, "the bait", will induce a complimentary charge or charges [amino acid residue(s)] in the binding pocket. The substrate, "the switch", will lack this charge, but will retain a similar overall structure. The monoclonal antibodies that bind these substrates now have in their combining sites strategically placed charged amino acid residues to accelerate the catalytic process. In this communication, we demonstrate how our "bait and switch" principle in an antibody-catalyzed cationic cyclization process allows for remarkable control of product selectivity.

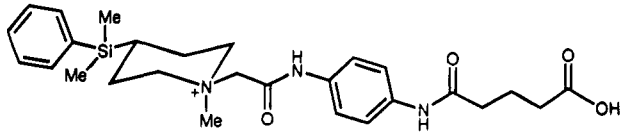
Cationic cyclization reactions are considered from both an enzymatic and a biosynthetic standpoint to be one of the most captivating chemical processes.[4] However, in spite of much effort, complete control of these reactions remain elusive. The success of polyene cyclization reactions can be traced to three critical steps: the initiation, the propagation, and the termination of the reaction.[4c] Among these steps, the stabilization and ultimate trapping of the cationic intermediate(s) generated is of prime importance. To date, there has been one example of an antibody-catalyzed cyclization reaction.[5] *N*-Oxide **1** (Chart 1) induces antibody catalysts that selectively transform the sulfonate ester **3** into the cyclohexanol adduct **5** (Scheme 1) via a π route cationic cyclization process. We now report that an alternative hapten can be used to dramatically alter the product outcome of the same reaction thus showing the precision with which antibody catalysis can control reaction trajectories.

The dative *N*-oxide bond of hapten **1** was envisioned as a mimic of the developing cationic carbon and anionic oxygen centers as the sulfonate leaves in the cyclization process.

Chart 1. Haptens Used To Generate Catalytic Antibodies for Cationic Cyclization Reactions

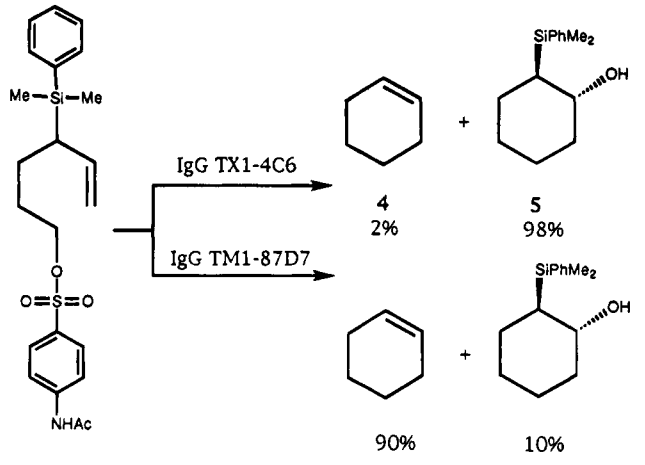


1



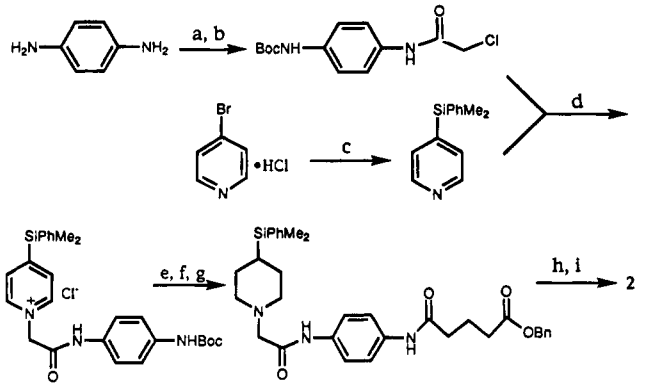
2

Scheme 1. Product Distribution from the Antibody-Catalyzed Reactions



3

Scheme 2. Synthesis of Hapten **2**[a]



[a] Conditions: (a) Boc_2O, 64%; (b) chloroacetyl chloride, 94%; (c) n-BuLi, chlorodimethylphenylsilane, 30%; (d) NaI (cat.), 70%; (e) H_2/PtO_2, 80%; (f) HCl/dioxane, 100%; (g) benzyl glutarate acid chloride, 82%; (h) MeI, 80%; (i) NaOH, 50%.

However, the *N*-oxide contains only partial charges, and we wondered about the implications of placing a full positive point charge in the vicinity where cleavage and hence cyclization are initiated. We anticipated that a quaternary ammonium ion could induce a binding pocket rich in aromatic amino acid residues which could effectively stabilized carbocations.[6] As such, hapten **2** was synthesized according to the scheme shown in Scheme 2 and coupled to carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). Balb/c nice were immunized with the KLH conjugate of **2**, and antibodies

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were generated by standard protocols.[7] Immunization of **2** produced 18 monoclonal antibodies. All antibodies were of the IgG class and were purified from ascites fluid by anion-exchange chromatography followed by affinity chromatography on a protein G column.[8] Antibodies were judged to be homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Antibodies at a concentration of 5 μM were initially screened using our biphasic solvent conditions (83% pentane/2% chloroform/15% Bis-Tris buffer, 50 mM, pH 7.0) and **3** at a concentration of 200 μM.[9] This preliminary high-performance liquid chromatography (HPLC) screen provided us with one antibody, TMI-87D7, that effectively cleaved the sulfonate ester of **3** ($k_{cat} = 0.02$ min^{-1}, $K_m = 25 \times 10^{-6}$ M).[10] To ascertain the nature of the terminating products, gas chromatography (GC) analysis was employed. The products observed from the 87D7-antibody-catalyzed reaction were cyclohexene (**4**) (90%) and *trans*-2-(dimethylphenylsilyl)cyclohexanol (**5**) (10%). The mass balance of these two products matched the production of 4-acetamidobenzenesulfonic acid.[11] The reaction is inhibited by the addition of **2** to the reaction mixture ($K_i = 1.4$ μM) thereby demonstrating that the reaction takes place in the combining site of the antibody.

Because of the extreme variances in product ratios observed between TX1-4C6 and TM1-87D7 (Scheme 1), NMR studies were engaged to see if there was an overall difference in the preferred conformations which **1** and **2** adopted in solution. The silicon appendage and the phenylenediamide linker in **1** and **2** were unambiguously assigned to equatorial positions based on nuclear Overhauser effect (NOE) measurements while the oxygen and methyl functionalities on **1** and **2**, respectively, were assigned to an axial orientation. On the basis of these findings, the only major difference between the two haptens is the charge distribution found at the quaternary nitrogen atom, and thus we would expect that antibodies induced to either hapten should bind both in a similar fashion.

The overall structural difference (vide supra) among haptens **1** and **2** is slight, yet, as shown in Scheme 1, an almost complete reversal in the product ratios from antibodies obtained to these two haptens was observed. On the basis of our NMR studies it is remarkable that such a significant amount of cyclohexene (**4**) is seen with IgG 87D7, especially considering the required pseudoaxial position which the silicon appendage in **3** must obtain before elimination can occur.

It is tempting to hypothesize that the differences in charge between these two haptens is the governing factor for the product differences seen between these two catalysts. To achieve the stereoelectronic requirements for the elimination of the silicon moiety we would suggest that much of the binding energy in the case of IgG 87D7 is directed toward charge stabilization. This allows more "relaxed" recognition of the silyl appendage[12] or an overall more "flexible" binding pocket thereby setting up an induced-fit mechanism of action in the antibodies' catalytic process.[13]

A rich history of over 40 years of scientific endeavors has been invested into polyene cyclization processes. Interestingly, given that much research effort has gone into this reaction class both biochemically and synthetically, the exact mechanism of action of polyene cyclization is still unknown.[14] In the context that these reactions can be viewed as a cascade of cationic intermediates, catalytic antibodies may be useful tools for exploring the nature of these biological catalysts.

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(7) Kohler, G.; Milstein, C. *Nature* **1975**, *256*, 495. The conjugate was prepared by slowly adding 2.5 mg of **3** in 250 μL of 0.01 M sodium phosphate buffer, pH 7.2, while stirring at 4 °C for 1 h. Each of four 8-week-old 129G1X$^+$ mice received in intraperitonal injection of 100 μg of **3** conjugated to KLH and RIBI adjuvant (MPL and TDM emulsion). A 50-μg intraperitoneal injection of **3**-KLH conjugate in alum was given 2 weeks later. One month after the second injection the mouse with the highest titer (12 800−25 600) was injected intravenously with 50 μg of **3**-KLH conjugate; 3 days later, the spleen was taken from the preparation of hybridomas. Spleen cells (1.0×10^8) were fused with SP2/0 (1.4×10^2) and HL myeloma cells (2.3×10^7). Cells were plated into 30 96-well plates; each well contained 150 μL of hypoxanthine, aminopterin, thymidine−Dulbecco's minimal essential medium (HAT−DMEM) containing 1% nutridoma, and 2% bovine serum albumin.

(8) The γ-globulin-containing fractions from ascitic fluid were precipitated by dropwise addition of saturated ammonium sulfate at 4 °C, pH 7.2, until a final concentration of 45% was achieved. The ammonium sulfate was removed by dialysis against 10 mM tris, pH 8. The concentrated antibodies were then purified by anion exchange chromatography on DEAE-Sephacel and eluted with a stepwise salt gradient (50−500 mM NaCl). The antibodies that eluted in the 100 mM NaCl fraction were concentrated by ultrafiltration before affinity purification on a protein G-Sepharose column. The antibody was loaded onto the column, and nonadherent material was removed by extensive washings (20−30 column volumes). The column was eluted with 0.05 M citric acid, pH 3.0, and fractions were immediately neutralized by collection into 1 M tris, pH 9.0. All antibodies were then concentrated and dialyzed into 50 mM BIS-Tris, pH 7.0, and assayed by HPLC or GC.

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(10) No background reaction could be detected under our assay conditions.

(11) Acyclic products resulting from the solvolysis of the sulfonate were not observed.

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