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First Example of an Antibody-Catalyzed Aza Diels–Alder Reaction

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Abstract—Described herein is the synthesis of a hapten of biocyclo[2,2,2]octene **5** designed to mimic the *exo* transition-state of an aza Diels–Alder reaction. Immunization of rabbits with this hapten provided polyclonal antibodies, Aza-BSA-3, which were used to synthesize adduct **4b** in the first reported antibody-catalyzed *exo* Diels–Alder reaction.

The Diels–Alder reaction is one of the most important carbon–carbon forming processes in organic chemistry. It is also a versatile tool for stereoselective synthesis of six-membered ring compounds.[1] The cycloaddition of a diene and a dienophile is a bimolecular process that has a large entropic barrier, with a typical activation entropy in the range of -30 to -40 cal K^{-1} mol^{-1} (1 cal = 4.184 J). To date, there is no unequivocal proof for the existence of natural Diels–Alderases, although plausible products from enzyme-catalyzed Diels–Alder reactions have been reported.[2] Using tailored catalysts from mammalian immune systems, several groups have successfully generated specific antibodies that catalyzed Diels–Alder reactions, including homo-Diels–Alder reactions[3] and hetero-Diels–Alder reactions.[4] Herein we reported the first example of an antibody-catalyzed aza Diels–Alder reaction.

The reaction selected for investigation was the cycloaddition of an unsymmetrical diene **1** to a chiral dienophile **2** (Scheme 1). This choice was based on the following reasons. (i) The cycloaddition of an aza dienophile to a diene is a very useful synthetic method. For example, through further oxidization of product double bonds, our designed reaction would offer a new facile synthesis of biologically active molecules, including analogues and homologues of aza glucose. (ii) Under the catalysis of a mixed acid (1 equiv CH_3SO_3H and 1 equiv CF_3CO_2H), reaction of the diene **1** with the dienophile **2** would predominantly produce the favored adduct **4a**, with the ratio of the *endo* adduct **4a** and the *exo* adduct **4b** being 4:1, whereas utilization of antibodies can reroute the reaction and afford selective formation of the disfavored *exo* adduct **4b**. (iii) The hapten of bicyclo[2,2,2]octene **5** was designed to mimic the *exo* boat-shaped transition-state **3b**, which shares the same relative and absolute carbon configurations.

Hapten **5** was initially synthesized via the route shown in Scheme 2. The required alcohol **6** was prepared from an allylic sulfone and ethylene epoxy according to literature procedures.[5] Protection of the primary alcohol **6** with iodomethane gave the methoxy ether **7**. The substrate diene **8** was prepared by reference to methodology developed by Bävckall.[5] Chiral dienophile **2** was synthesized as reported.[6] A key reaction step, the asymmetric aza Diels–Alder reaction of cyclohexadiene **8** with the chiral dienophile **2,** was subsequently carried out, producing only the *exo* adduct **9** under mixed protic acid catalysis (1 equiv CF_3CO_2H and 1 equiv CH_3SO_3H). The desired hapten **5** was obtained following several additional steps, including hydrolysis of the ethyl ester of compound **9** and condensation with methyl γ-amino butylrate, followed by hydrolysis of the methyl ester of compound **11**.[7]

Immunogens **12** and **13** were prepared by coupling carrier protein Bovine Serum Albumin (BSA) or Flow γ

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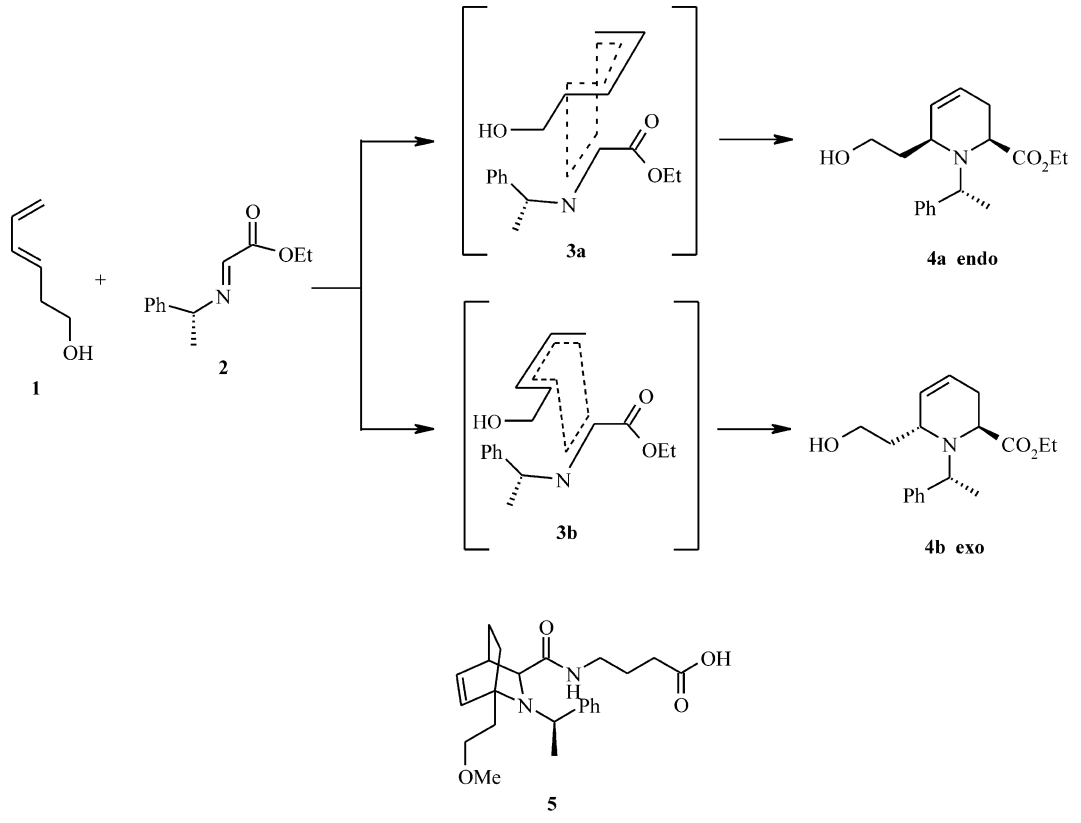
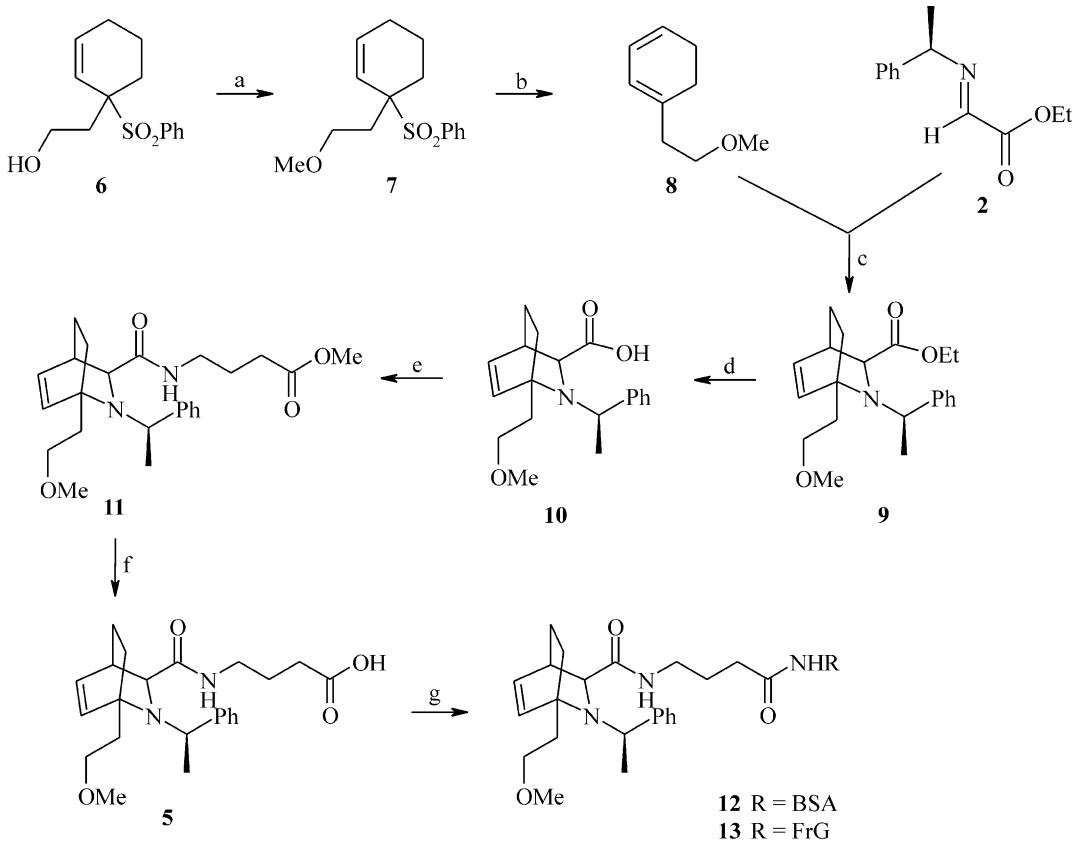


Scheme 1. The antibody-catalyzed aza Diels–Alder reaction and design of the transition-state analogue **5**.



Scheme 2. Reagents and conditions: (a) Ag_2O, $CaSO_4$, MeI, rt, 3 days, 100%; (b) ^{t}BuOK, ^{t}BuOH, reflux, 8 h, 84%; (c) CF_3CO_2H (1 equiv), CH_3SO_3H (1 equiv), CH_2Cl_2, −78 °C 2 h to rt overnight, 67%; (d) 2 N NaOH, 90% EtOH, 60 °C, overnight, 89%; (e) $HCl{\cdot}H_2N(CH_2)_3CO_2Me$, *N*-methyl morpholine, 2-chloro-4,6-dimethoxy-1,3,5-triazine, DMF, rt, 24 h, 86%; (f) 2 N NaOH, 90% MeOH, 60 °C, 3 h, 90%; (g) EDCI, DMF, 10 mmol/L PBS buffer (pH 7.2), rt, 5 h.

Globin (FγG) in PBS (pH 7.2) using 1-(3-dimethylaminopropyl)-3-ethyl carboimide hydrochloride (EDCI) as condensation reagent. The immunized rabbits were bled after 1-month post-immunization and the sera were precipitated using saturated ammonium sulfate and separated by ion exchange (DE-52) to remove undesired serum proteins.[8]

By this procedure four polyclonal antibodies were acquired. Kinetic experiments demonstrated that one polyclonal antibody, Aza-BSA-3, could catalyze the desired aza Diels–Alder reaction. The rate of the reaction was measured by monitoring the disappearance of the diene at 242 nm using reversed-phase high performance liquid chromatography (HPLC). The catalyzed reaction was performed under conditions of: diene **1** (370 μM); dienophile **2** (4000 μM); polyclonal antibody (7.4 μM), 37 °C at pH 7.0 in a PBS (10 mM) buffer. Initial rates of catalyzed reaction in the presence of antibodies were measured within 5% completion of the diene and corrected for background reaction in the absence of antibody. The data so obtained were employed to construct a Lineweaver–Burk plot, from which the kinetic parameters were derived (Fig. 1).

Because dienophile **2** was in excess, the results were observed to follow Michaelis–Menten kinetics of pseudo-first-order reactions, where K_M/V_{max} and $1/V_{max}$ are respectively determined as the slope of the line and the intercept of the vertical axis in Figure 1. The values of kinetic parameters for the diene **1** were: $K_M = 833$ μM, $V_{max} = 1.82$ μM/min, $k_{cat} = 0.34$ min^{-1}.

According to our experiments, the polyclonal antibody accepted as substrates the diene **1** and the dienophile **2** and produced the expected adduct **4b**. The ratio of the *exo* adduct **4b** to *endo* adduct **4a** was 13:1 under the catalysis of Aza-BSA-3, while the ratio of *exo* adduct to *endo* adduct was 1:4 under the catalysis of mixed protic acid (1 equiv CF_3CO_2H and 1 equiv CH_3SO_3H).[9] However, neither *exo* nor *endo* adduct could be detected if the reaction was run without any catalysts. Addition of an equimolar amount of inhibitor **10** to antibody-catalyzed reactions resulted in complete inhibition, with the reaction rate dropping to the background value. This indicated that catalysis took place utilizing antibody binding sites. Controls were performed under the same conditions using non-specific rabbit immunoglobulin G, which showed no influence on the reaction rate.

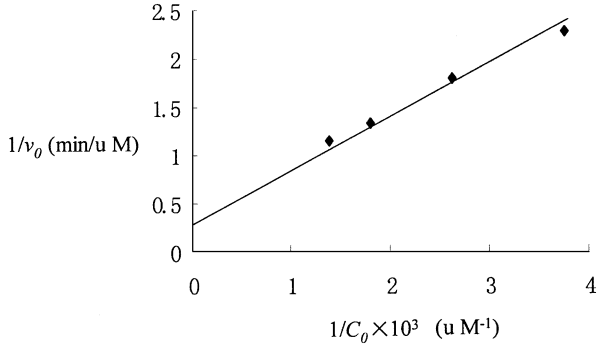


Figure 1. Lineweaver–Burk plot for the reaction of diene **1** with dienophile **2** catalyzed by Aza-BSA-3.

In summary, herein we report the first antibody-catalyzed aza Diels–Alder reaction through an extension of our previous study.[10] Further kinetic analysis and exploration of enantioselectivity under catalysis of monoclonal antibodies are under way.

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7. All new compounds gave satisfactory spectral and microanalytical data. Selected data for compound **9**: $[\alpha]_D^{20} = -95.2$ (*c* 1.2, MeOH); IR (film, cm^{-1}): 3031, 2976, 2875, 1747, 1497, 1451, 1375; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.19 (m, 5H, –Ph), 5.97 (m, 1H), 5.87 (d, $J = 8.0$ Hz, 1H), 4.24 (q, $J = 7.2$ Hz, 2H), 4.15 (q, $J = 7.1$ Hz, 1H), 3.47 (t, $J = 7.4$ Hz, 2H), 3.46 (m, 1H), 3.31 (s, 3H), 2.73 (m, 1H), 2.34 (m, 1H), 2.05 (m, 1H), 1.90 (m, 1H), 1.61 (m, 1H), 1.32 (d, $J = 7.2$ Hz, 3H), 1.21 (t, $J = 7.3$ Hz, 3H), 1.03 (m, 1H), 0.92 (m, 1H); MS (EI) *m*/*z* 343 $(M)^+$, 105 (100); HRMS calcd for $C_{21}H_{29}O_3N$ $(M)^+$: 343.2161; found: 343.2148. Compound **11**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (t, $J = 6.0$ Hz, 1H), 7.26–7.18 (m, 5H, –Ph), 5.83 (m, 1H), 5.65 (d, $J = 7.8$ Hz, 1H), 4.32 (q, $J = 7.2$ Hz, 1H), 3.71 (s, 3H), 3.60–3.53 (m, 2H), 3.44–3.41 (m, 2H), 3.39 (s, 3H), 3.35 (m, 1H), 2.86 (m, 1H), 2.42 (t, $J = 7.2$ Hz, 2H), 2.32 (m, 1H), 2.21 (m, 1H), 1.92 (t, $J = 7.2$ Hz, 2H), 1.70 (m, 1H), 1.51 (m, 1H), 1.30 (d, $J = 6.6$ Hz, 3H), 1.21 (dt, $J = 3.0$ Hz, $J = 7.3$ Hz, 1H), 0.99 (m, 1H); ^{13}C NMR (120 MHz, $CDCl_3$) 175.0, 173.5, 142.2, 136.7, 130.6, 129.0, 127.8, 127.0, 69.1, 61.4, 58.8, 56.7, 56.5, 51.7, 38.4, 36.0, 33.4, 32.2, 31.6, 25.1, 20.8, 20.3; MS (EI) *m*/*z* 414 $(M)^+$, 105 (100). Anal. calcd for $C_{24}H_{34}O_4N_2$: C, 69.57, H, 8.21, N, 6.76; found: C, 69.40, H, 8.10, N, 7.02. Compound **5**: $[\alpha]_D^{20} = -120.1$ (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (t, $J = 6.0$ Hz, 1H), 7.26–7.17 (m, 5H, –Ph), 5.78 (m, 1H), 5.66 (d, $J = 8.4$ Hz, 1H), 4.25 (q, $J = 7.2$ Hz, 1H), 3.55 (m, 1H), 3.50 (m, 1H), 3.31 (s, 3H),

3.20 (m, 1H), 3.14 (brs, 1H), 2.62 (m, 1H), 2.26 (t, $J=8.4$ Hz, 2H), 2.21 (m, 1H), 2.13 (m, 1H), 1.78 (m, 1H), 1.72 (m, 2H), 1.39 (m, 1H), 1.23 (d, $J=7.2$ Hz, 3H), 1.11 (dt, $J=3.0$ Hz, $J=6.6$ Hz, 3H), 0.85 (m, 1H); MS (EI) m/z 400 $(M)^+$, 105 (100). Anal. calcd for $C_{23}H_{32}O_4N_2{\cdot}2.5H_2O$: C, 62.02, H, 8.31, N, 6.29; found: C, 62.29, H, 7.95, N, 6.01.

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9. HPLC measurements were performed using a C-18 reverse-phase column, employing isocratic conditions: CH_3CN/H_2O 2.5:7.5, with UV detection at 242 nm, retention time for product **4a** 25.444 min and for product **4b** 27.048 min.

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