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Polyclonal Catalytic Antibody for Hetero-Cycloaddition of Hepta-1,3-diene with Ethyl Glyoxylate An Approach to the Synthesis of 2-Nonulosonic Acid Analogs

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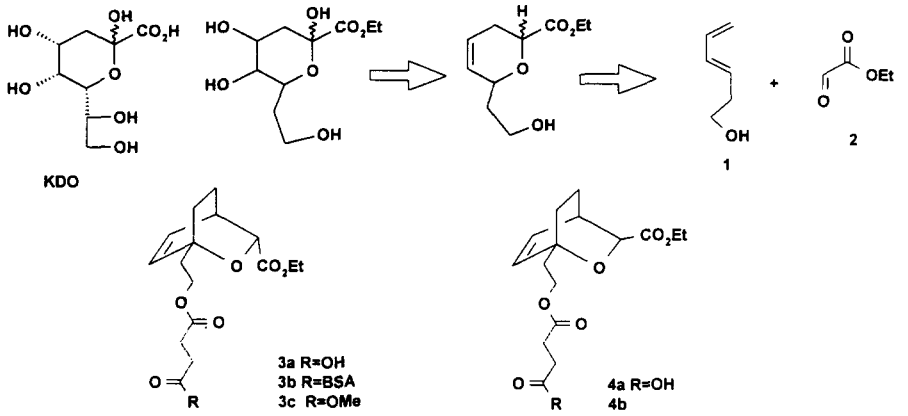
Abstract: Polyclonal antibody raised from a product analogue for a Diels-Alder reaction has been found to catalyze the planned *endo*-cycloaddition between ethyl glyoxylate hydrate and diene, adduct of which would be useful for the synthesis of ulosonic acid and its analogs.

It has been ten years since the first examples of catalytic antibodies were reported in the literature. During this decade significant progress has been made in the development of hapten design, antibody generation, and methods of screening for catalysis.[1]

The Diels-Alder transformation is one of the most important carbon-carbon bond formation process in chemistry.[2] However, up to now there is no unequivocal proof for the existence of a Diels-Alderase, although circumstantial evidence from the discovery of intermediate metabolites containing dienedienophile functionality and of the plausible products of enzyme-catalyzed Diels-Alder reaction has been reported[3]. To fill up the vacancy in the nature, Hilvert developed antibody that catalyzed the Diels-Alder reactions.[4] Other Diels-Alder antibody catalysts[5] have been reported by Braisted, Schultz, Suckling et al., whilst Meekel et al.[6] have revealed the first example of an antibody catalyzed-hetero Diels-Alder reaction. Their work prompts us to report our studies on antibodies that catalyzed the Diels-Alder reactions, which might be a key step in the

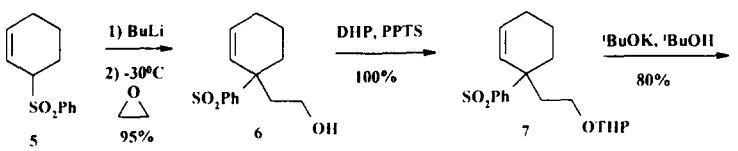
syntheses of some natural products, such as the biologically important ulosonic acid (e.g. 3-Deoxy-D-manno-2-octulosonic acid (KDO)[7]).

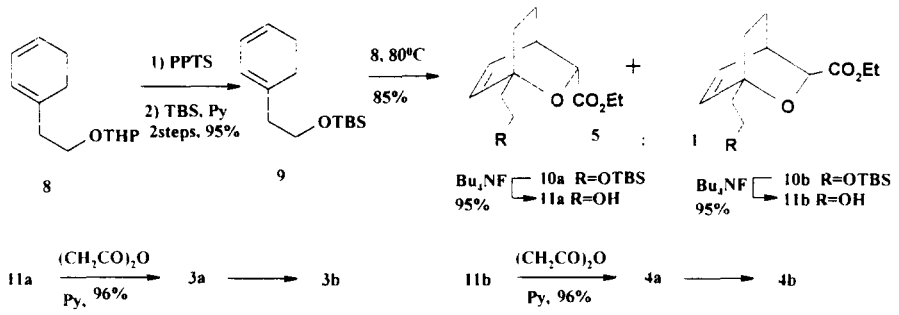
Recently, based on the aqueous hetero Diels-Alder reaction of water-soluble diene and ethyl glyoxylate, Lubineau et al. have developed a new straightforward strategy for the synthesis of ulosonic acid family.[8] Unfortunately, both the yield and the selectivity of these hetero Diels-Alder reactions were not satisfied. Therefore we tried to use antibody as the stereoselective catalyst for this kind of reactions. As the first step, achiral diene and ethyl glyoxylate were chosen as the substrate and bridge compound **3a** and **4a** were designed as the hapten (Scheme 1).



Scheme 1 Retrosynthetic analysis of KDO analog and the bridge compounds designed as the hapten and antigen.

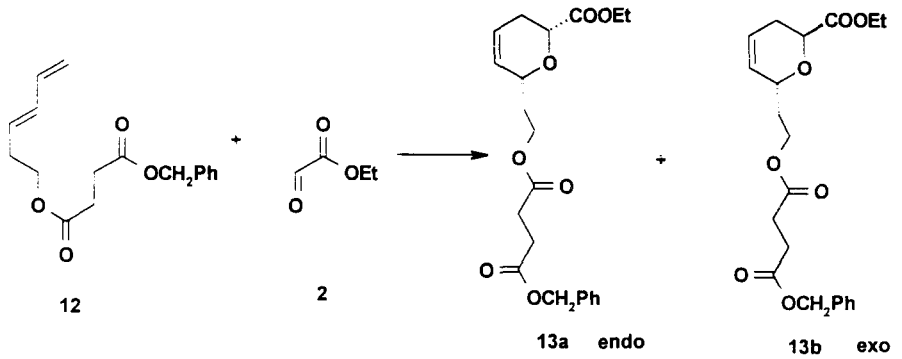
Hapten **3a** has been synthesized according to the route shown in Scheme 2. The required alcohol **6** was prepared in racemic form from sulfone **5** and epoxyethane according to the published procedure.[9] Diene **8** was synthesized from sulfone **7** using a methodology developed by Bäckvall.[10] Temporary blocking of the primary alcohol **6** proved necessary to achieve a clean 1,4- elimination of benzenesulfinic acid without concomitant isomerization of the diene moiety. After changing the protective group, diene **9** was obtained. The hetero-Diels-Alder reaction of cyclohexadiene with ethyl glyoxylate **2** produced an 5:1 mixture of 2-endo and 2-exo adducts (**10a** and **10b**). Desilylation with tetrabutylammonium fluoride and coupling of **11a** and **11b** with succinic anhydride gave hapten **3a**, **4a**[11], respectively.





Scheme 2

Hapten **3a** was conjugated to carrier proteins bovine serum albumin (BSA) by using 1-(3-dimethylamino-propyl) -3-ethylcarbodiimide hydrochloride. Antibodies were generated by standard protocols and purified from the whole serum by ammonium sulfate precipitation(SAS), anion exchange chromatography on DE-52 cellulose and were determined to be >95% homogeneous by capillary electrophoresis.[12]



Scheme 3 Diels-Alder reaction by polyclonal antibody catalysis.

For the kinetic measurement of the hetero-Diels-Alder reaction diene **12** was used as the dienophile substrate, in which a benzyl ester chain was attached the facility of detection by UV. The cycloaddition between diene **12** (10μM) and ester hydrate **2** (200μM) by the polyclonal antibody (0.7μM) was assayed at 37°C at pH 6.5 in a 100mM NaCl PBS buffer and monitored by reversed-phase high performance liquid chromatography (RP-HPLC). The polyclonal antibody accepted both diene and ethyl glyoxylate hydrate as substrates and produced the expected adduct, whereas in the absence of antibody no cycloaddition reaction could be detected at all.

Reaction carried out in the presence of antibodies followed Michaelis Menten kinetics. The Lineweaver Burk plots gave the following data: K_M = 96 μM for diene **12**, v_{max} = 0.98 μM / min.. The polyclonal antibody obtained against hapten **3b** showed moderate rate enhancement and the further characterization is under way.

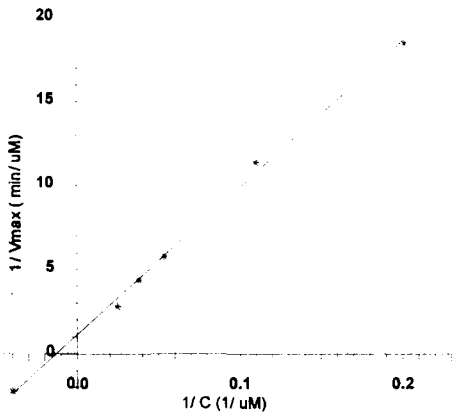


Figure 3 Lineweaver-Burk plot of polyclonal antibody catalyzed Diels-Alder reactions between Diene **12** and ethyl glyoxylate **2** (0.5μM polyclonal antibody IgG, 100mM NaCl, PH=6.5). Velocities were determined at 37°C by monitoring the formation of adducts with an HPLC assay (versed-phase C-18 column, 25% water and 75% acetonitrile). Product **13a**, detected at 214nm, was identified by coinjection with an authentic sample.

Addition of an inhibitor, the bridge compound **3c** (96μM), to the antibody-catalyzed reactions resulted in complete inhibition, with the rate dropping to the background value (the cycloaddition rate was too small to be detected even at prolonged time to 48 h). This revealed that catalysis took place at the antibody binding sites. Under the same conditions, controls were performed by using normal rabbits immunoglobulin G, which showed no influence on the reaction rate.

The products of cycloaddition between diene **12** and ester **2** were checked with the synthesized samples[13] **13a** and **13b** by HPLC. The polyclonal antibody derived from immunization against antigen **3b** was found to catalyze exclusively the formation of the *endo* adduct **13a**.

This work presents an extension of polyclonal antibody's catalysis in ester hydrolysis[12,14] to cycloaddition reactions. Further kinetic analysis and exploration of the enantioselectivity of this polyclonal abzyme are under way.

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11. All physical data are consistent with their structure. Their ^{1}H NMR spectra are shown here: **3a**: δ 6.70-6.20 (2H, m), 4.24 (2H, q, J=7.1Hz), 4.10 (1H, m), 3.87 (1H, m), 2.96 (4H, s), 1.24 (3H, t, J=7.1Hz); **3c**: ^{1}HNMR (300MHz, $CDCl_3$): δ 6.38 (1H, dd, J=8.3, 1.3Hz), 6.25 (1H, dd, J=8.3, 6.3Hz), 4.36 (2H, dt, J=7.3, 1.4Hz), 4.31 (1H, d, J=1.9Hz), 4.10 (2H, q, J=7.1Hz), 3.68 (3H, s), 3.08 (1H, m), 2.63 (4H, s), 2.18-1.98 (2H, m), 1.93-1.58 (2H, m), 1.22 (3H, t, J=7.1Hz); **4a**: δ 6.55-6.10 (2H, m), 4.60-4.25 (2H, m), 4.10 (2H, q, J=7.1Hz), 2.63 (4H, s), 2.30-1.60 (5H, m), 1.25 (3H, t, J=7.1Hz).
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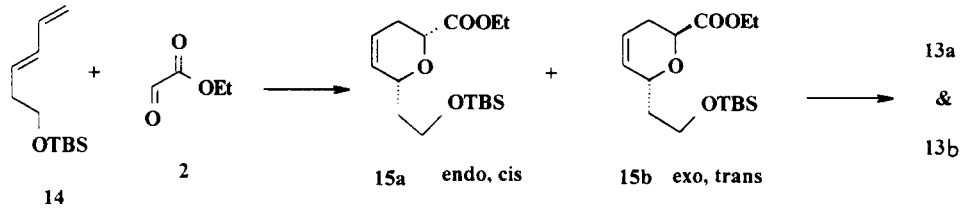
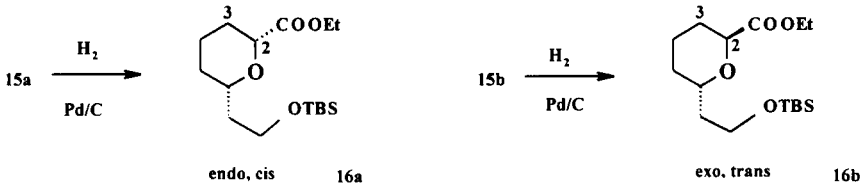
13. Authentic samples **13a** and **13b** couldn't be synthesized by thermal cycloaddition of **12** with **2**. Therefore, an indirect synthesis was made from the cycloaddition of hexa-3,5-dienol *tert*-butyldimethylsilyl ether, albeit the yield was also poor (20%). The crude adduct could be separated to endo-(cis)-adduct **15a** and exo-(trans)-adduct in ratio of 55/45, and then desilylation followed by esterification gave **13a** and **13b**, respectively.



The stereochemistry of the isomers **13a** and **13b** based on the coupling constants of compound **16a** and **16b** (**16a** $J_{2,3}$=11.6, 2.3 Hz; **16b** $J_{2,3}$=5.6, 2.5 Hz). **16a** and **16b** were prepared by hydrogenation of **15a** and **15b**, respectively.



^{1}H NMR spectra were recordered at 300MHz in $CDCl_3$ as solvent. Compound **13a**: δ 7.36 (5H, m), 5.87 (1H, m), 5.66 (1H, m), 5.13 (2H, s), 4.23 (6H, m), 2.64 (4H, m), 2.33 (2H, m), 1.90 (2H, m), 1.25 (3H, t, J=7.1Hz); Compound **13b**: δ 7.35 (5H, m), 5.88 (1H, m), 5.64 (1H, m), 5.10 (2H, s), 4.23 (6H, m), 2.64 (2H, s), 2.62 (2H, s), 2.34 (2H, m), 1.96 (2H, m), 1.24 (3H, t, J=7.1Hz); Compound **16a**: δ 4.13 (2H, q, J=7.1Hz), 3.92 (1H, dd, J=11.6, 2.3Hz), 3.77 (2H, m), 3.51 (1H, m), 1.82 (1H, m), 1.61 (2H, m), 1.22 (3H, t, J=7.1Hz), 1.02 (9H, S), 0.04 (6H, S); Compound **16b**: 4.47 (1H, dd, J=5.6, 2.5Hz), 4.14 (2H, q, J=7.1Hz), 4.03 (1H, t, J=6.5Hz), 3.83 (1H, m), 3.73 (1H, t, J=6.5Hz), 1.93 (1H, m), 1.83-1.23 (3H, m), 1.20 (3H, t, J=7.1Hz), 0.89 (9H, s), 0.04 (6H, s).

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