Selective Antibody-Catalyzed Solvolysis of *endo*-2-Norbornyl Mesylate

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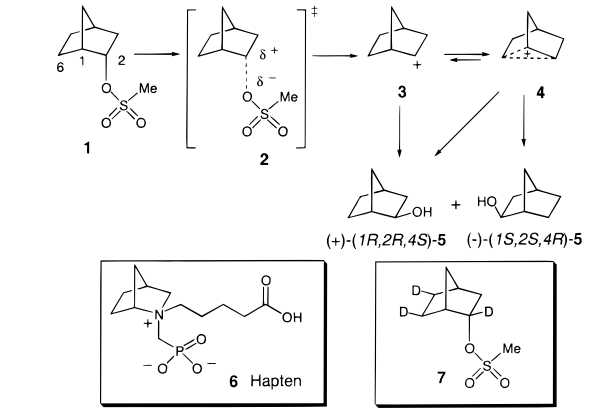
Extensive experimental[1] and computational data[2] have now firmly established that *endo*-2-norbornyl mesylate **1** undergoes solvolysis through an ionization process to initially form an unsymmetrical localized (classical) cation **3**, which is converted by the participation of the C1−C6 sigma bond to the more stable nonclassical cation **4**. The nonclassical cation is calculated to be more stable than the classical cation by about 6 kcal/mol.[3] The bridged intermediate **4**, involving delocalization of σ electrons, has a plane of symmetry and is achiral. Attack by water at C-1 or C-2 results in equal amounts of the enantiomeric *exo*-norbornyl alcohols **5** (Scheme 1).[4] The norborneol product is exclusively *exo*[5] since reaction occurs from the direction opposite of that of the bridging interaction. The rate-determining step for the solvolysis reaction involves the formation of transition state **2**.[6] Antibodies[7] elicited to an analogue of this transition state might be expected to sufficiently stabilize the initially formed localized cation **3** relative to the nonclassical ion **4** or slow the conversion of the former to the latter, such that reaction predominantly occurs from the classical cation **3**. Alternatively, the asymmetrical antibody combining site could differentiate the C-1 and C-2 positions of the nonclassical ion **4** so that they are attacked by nucleophile with unequal facility, resulting in enantiomerically enriched *exo*-2-norborneol. In either case, the antibody-catalyzed reaction would give products characteristic of the "classical" norbornyl cation.

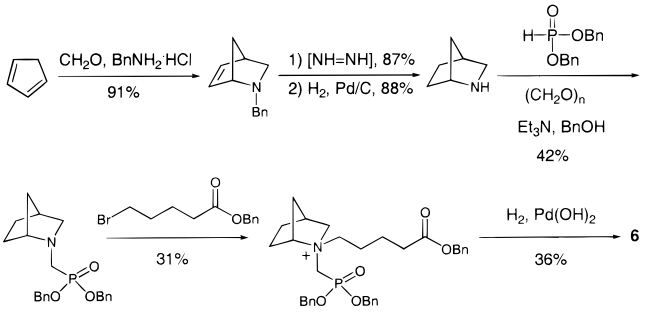
To test this notion, we generated antibodies to an analogue (**6**) of transition state **2** that mimics both the developing positive charge on the C-2 position and the developing negative charge of the sulfonate leaving group.[7,8] The synthesis of racemic hapten **6** was carried out as shown in Scheme 2; hapten **6** was linked to the carrier proteins keyhole limpet hemocyanin and bovine serum albumin via a five carbon linker. A total of sixteen monoclonal antibodies specific for **6** were obtained by standard methods.[9] Twelve of the antibodies were found by ELISA assays to bind racemic hapten **6** and were subsequently purified by protein G affinity chromatography and analyzed for catalytic activity. The solvolysis of racemic substrate **1** was carried out with or without antibodies at 22 °C in aqueous 10 mM phosphate, 100 mM NaCl, pH 7.4 buffer (PBS). The reaction mixture was quenched by rapid extraction with ethyl acetate and quantitatively assayed by gas chromatography (GC)[10] with toluene as the internal standard. One antibody (15M3) showed a significant rate enhancement over the uncatalyzed reaction in an initial screen for 2-norborneol product. Only the *exo*-product was isolated, and no *endo*-2-norborneol was detected.[10] This result is consistent with a C−O versus S−O bond cleavage mechanism.[11] Antibody 15M3 was further purified by ion-exchange (Mono Q) chromatography and characterized in detail.

The first-order rate constants and kinetic parameters for both the uncatalyzed and antibody-catalyzed reactions were derived from the initial velocities by using the computer program

Scheme 1



Scheme 2



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(10) Gas chromatographic separation of the product mixture of solvolysis reactions was carried out on a methyl silicone capillary column (HP-1, 25 m × 0.2 mm) with a Hewlett-Packard 5890 Series II gas chromatograph. GC conditions: injector temperature = 245 °C, detector temperature = 250 °C, oven temperature, initially at 50 °C for 6 min and then raised to 200 °C at 20 °C/min. The retention times for *exo*-2-nornorneol **5**, *endo*-norbornyl mesylate **1**, and the internal standard toluene are 8.9, 13.1, and 2.9 min, respectively. *Endo*-2-norborneol (Aldrich) has a retention time of 9.3 min under these conditions. All compounds are stable under these conditions, and no decomposition of materials was observed.

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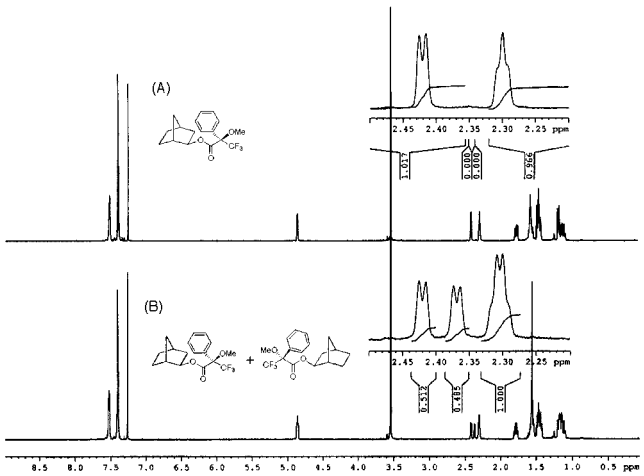

Figure 1. Comparison of the product distribution from the antibody-catalyzed and uncatalyzed solvolysis reactions: (A) 500 MHz ^{1}H NMR spectrum (in $CDCl_3$) of the (*R*)-Mosher's ester from *exo*-2-norborneol isolated from the antibody-catalyzed solvolysis of racemic **1** and (B) 500 MHz ^{1}H NMR (in $CDCl_3$) of the (*R*)-Mosher's ester from *exo*-2-norborneol isolated from the uncatalyzed solvolysis of **1**. Insets are expanded signals of the nonequivalent 1-hydrogen and 4-hydrogen groups[19] in the norbornyl moiety for diastereomeric (*R*)-Mosher's esters.

DYNAFIT.[12] The background rate of solvolysis of mesylate **1** was determined to be 9.0×10^{-4} min^{-1} at 22 °C in PBS buffer. Antibody-catalyzed solvolysis of **1** was found to follow Michaelis−Menten kinetics with a k_{cat} of 1.7 min^{-1}, a K_m of 1.5 mM, and a resulting k_{cat}/k_{uncat} of 1900. Furthermore, the antibody-catalyzed reaction was inhibited by hapten **6**: least-squares Dixon analysis afforded a K_i value of 9.2 ± 0.5 μM. The K_i value is significantly lower than that of the K_m, indicating that the antibody preferentially binds to the charged transition-state analogue **6** relative to substrate **1**. The conversion rate of enantiomerically pure (1*S*,2*R*,4*R*)-**1**[13] to *exo*-2-norborneol at 22 °C in the presence of 14 μM antibody 15M3 was identical to the rate of the background reaction, demonstrating that only the (1*R*,2*S*,4*S*)-**1** enantiomer is a substrate for the antibody-catalyzed reaction. The antibody-catalyzed reaction was found to be insensitive to pH variations (pH 6.5−8.5).

To investigate the stereoselectivity of the solvolysis reaction, we followed the spontaneous solvolysis of racemic substrate **1** to completion and isolated the resulting product and subsequently converted it to Mosher's ester[14] by reacting the formed *exo*-2-norborneol product with (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride. The *exo*-2-norborneol derived from the uncatalyzed solvolysis reaction was found to be an equimolar mixture of two diastereomers as shown by its ^{1}H NMR spectrum (Figure 1B), indicating a racemic mixture of *exo*-2-norborneol **5** formed by solvolysis of **1**. The antibody-catalyzed solvolysis reaction of racemic **1** was carried out at 22 °C in the presence of 30 μM antibody 15M3 and terminated (<40% conversion) by extraction of *exo*-2-norborneol product with ethyl acetate. After purification by flash chromatography on silica gel (elution with 10% ethyl acetate in hexane), this product was converted to its Mosher's esters and determined by NMR analysis to be 96% enantiomerically pure. The enantiomeric enrichment of *exo*-2-norborneol from the antibody-catalyzed reaction was further confirmed by the determination of optical rotations. While the product from the uncatalyzed solvolysis reaction displays no optical activity, the specific rotation $[\alpha]^{20}$ for the *exo*-2-norborneol from the antibody-catalyzed reaction was determined to be + 2.97° ($CHCl_3$, *c* 0.5). In comparison to the reported specific rotations[15] for (+)-(1*R*,2*R*,4*S*)-**5** {$[\alpha]^{25} = +3.06°$ ($CHCl_3$, *c* 3)} and (−)-(1*S*,2*S*,4*R*)-**5** {$[\alpha]^{25} = -3.14°$ ($CHCl_3$, *c* 3.1)}, respectively, the antibody-catalyzed solvolysis reaction of racemic **1** afforded (+)-(1*R*,2*R*,4*S*)- and (−)-(1*S*,2*S*,4*R*)-**5** (98:2 ratio).

To determine whether the antibody-catalyzed reaction occurs through an S_NL or S_N2 substitution mechanism, we synthesized a trideuterated substrate (**7**). The kinetic isotope effect $^{\alpha-D}(V/K)/^{\alpha-H}(V/K)$ was measured by direct determination of the ratios of labeled to unlabeled substrate and product in a mixture by mass spectroscopy.[16] The observed isotope effect for the uncatalyzed solvolysis reaction is 1.15 ± 0.03, while the antibody-catalyzed (PBS, 22 °C) reaction affords an isotope effect of 1.33 ± 0.04, based on four determinations. A secondary isotope effect of 1.15 for the uncatalyzed reaction is consistent with formation of a nonclassical ion (Scheme 1).[17] The kinetic isotope effect of 1.33 for the antibody-catalyzed solvolysis reaction is also consistent with an S_N1-type mechanism, but one in which greater rehybridization occurs at the 2 position of substrate **1** relative to the uncatalyzed reaction, possibly due to enhanced stabilization of the classical cation.

Antibody 15M3 not only shows a high degree of substrate specificity; it also yields products characteristic of the classical 2-norbornyl cation. This might arise from selective stabilization of the cation relative to the nonclassical ion in an asymmetric antibody combining site. Alternatively, the antibody might slow the rate of conversion of the initially formed C-2 cation to the nonclassical ion relative to the rate of trapping by solvent. If the antibody-catalyzed solvolysis reaction is occurring through to the nonclassical cation intermediate, then the trajectory of solvent attack is controlled such that reaction is favored at the C-2 position of the achiral nonclassical cation (**4**).[18] In any case this study demonstrates that antibodies can control the energetics of complex reaction coordinates to a remarkable degree.

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(16) To obtain sufficient signal-to-noise, we purified the 2-*exo*-norborneol products from an equimolar mixture of substrate **1** and substrate **7** and subsequently converted them to the corresponding higher molecular weight 4-nitrobenzoate esters.

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