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Design and Synthesis of Chiral and Racemic Phosphonate-Based Haptens for the Induction of Aldolase Catalytic Antibodies

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Abstract—A novel strategy for the generation of aldolase catalytic antibodies, based on the use of antibody-catalyzed enol ester hydrolysis as a 'trigger' to generate a reactive enolate intermediate, is described. A model system to test this strategy was developed and substrate **8** was synthesized. However, the targeted bifunctional haptens **11** and **33** were synthetically inaccessible, and therefore the alternative phosphonate hapten **39** was prepared. The key step in the synthesis of **39** was the direct generation of an unprotected phosphonate precursor via coupling of the secondary alcohol **37** with $CH_3P(O)Cl_2$. The chiral counterpart of hapten **39** was also synthesized from alcohol **46**, prepared by Corey's asymmetric reduction method. One polyclonal antibody preparation generated from **39** appeared to catalyze the hydrolysis of the secondary acetate **49**, but not the desired aldol cyclization of **8**. Possible rationales for this finding are discussed. © 1997 Elsevier Science Ltd.

Introduction

One of the major goals in the field of catalytic antibodies[1–3] has been to use the understanding of reaction mechanisms to design new catalysts for synthetically useful reactions.[4] The generation of new carbon–carbon bonds is the central act in synthetic organic chemistry. Most of the catalytic antibodies described thus far catalyze acyl transfer reactions, and the majority of those that catalyze carbon–carbon bond formation accelerate pericyclic reactions.[5] The aldol reaction is one of the most powerful methods for the stereoselective formation of carbon–carbon bonds, and is widely used in the synthesis of sugars, polyhydroxy antibiotics, and many other biologically active compounds.[6] One of the potential advantages of enzymes (and catalytic antibodies) is their ability to catalyze chemo-, regio-, and stereoselective reactions without the need for protecting groups. Aldolases are an important class of enzymes that are especially important for carbohydrate biosynthesis.

Recent advances have been made in the synthetic use of aldolases,[7] such as the tandem aldol condensations catalyzed by 2-deoxyribose-5-phosphate aldolase.[8] In this case, excellent stereochemical control has been achieved by the enzyme, which gives access to optically pure aldol products on a preparative scale. A limitation of aldolases is their high specificity for their carbanion component, the aldol donor. In general, there are only four compounds from which the enzyme-bound enolate equivalent is received: dihydroxyacetone phosphate, pyruvate, phosphoenolpyruvate, and acetaldehyde. The aldol reaction is a natural target for antibody catalysis, but only three recent examples of aldolase antibodies have been reported.[9] Reymond and Chen have reported that an antibody can utilize an amine cofactor to catalyze an aldol reaction.[10] A second antibody induced by hapten **3** catalyzes the enolization of the ketone moiety in substrate **1**, which then cyclizes to **2** (Fig. 1A).[11] Note that the antibody does not catalyze the actual carbon–carbon bond formation step. A third class of aldolase antibodies was recently described by Wagner et al.[12] Certain antibodies generated by the hapten **7** possess a lysine group in their combining site that reacts with ketones to give the enamine intermediate **5** (Fig. 1B). This intermediate then reacts with aldehydes to give the aldol product **6**. Note that this sequence mimics the natural mechanism of class I aldolases.

Our objective was to develop a new class of aldolase antibodies, which would exploit a different paradigm for the generation of carbon–carbon bond-forming catalytic antibodies. We chose the intramolecular aldol condensation shown in Figure 1C as a model reaction. Hydrolysis of enol acetate **8** in the combining site of an antibody should give enolate **9**. This reactive intermediate could be stabilized through electrostatic or hydrogen bonding interactions within the antibody binding pocket. Enolate **9** should then rapidly react with the adjacent aldehyde functionality through a favored six-membered ring transition state to afford **10**. Compound **10** will undergo further elimination and tautomerization to 1-naphthol. Once the enolate is formed, carbon–carbon formation to give the aldol product should be rapid. Hence, our strategy is to use the hydrolysis of the enol ester as a 'trigger' to generate the reactive intermediate **9** and initiate the intramolecular aldol condensation. This proposal has enzymatic precedents, as the enzymes phosphoenolpyruvate

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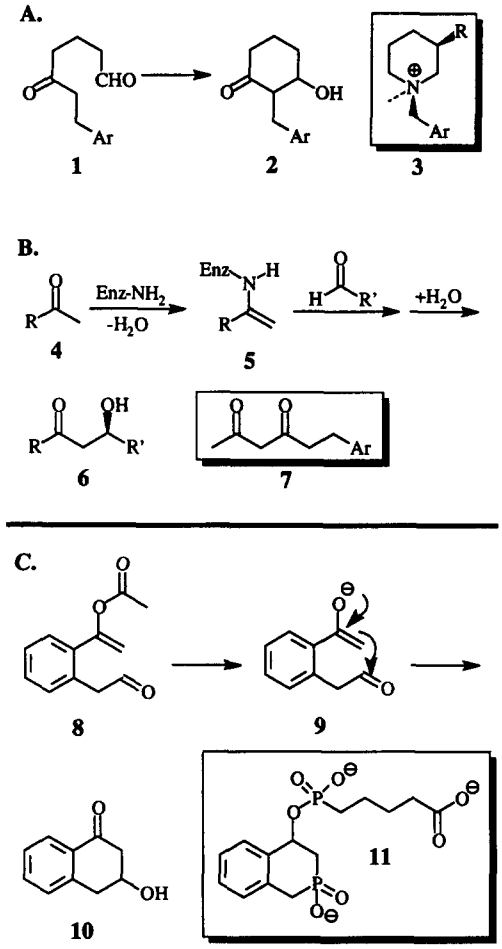

Figure 1.

carboxylase[13] and 3-dihydroquinate synthase[14] appear to generate transient, reactive enolate intermediates in their active sites. Note also that it has been demonstrated by Fujii and co-workers that an enol ester can be hydrolyzed by an antibody,[15] and the presumed enolate intermediate undergoes selective protonation in the antibody combining site.

The bicyclic phosphinate/phosphonate **11** appears to be an appropriate hapten to induce catalysts for the aldol cyclization of **8**. Since the hydrolysis of an enol ester bond is the initiating step in this intramolecular aldol reaction, the incorporation of a negatively charged, tetrahedral phosphonate group that mimics the transition state for enol acetate hydrolysis is essential. A five-carbon linker, which would be used for coupling to the carrier protein, is attached to the phosphonate center. The bicyclic ring would restrict rotational degrees of freedom in the formation of an aldol product. The phosphinate group at 2-position of the bicyclic ring resembles the transition state of the aldehyde carbonyl undergoing attack by the enolate. We anticipated that this hapten would induce bifunctional catalytic antibodies;[11,16] that is, antibodies that can catalyze both enol acetate hydrolysis and the subsequent aldol condensation.

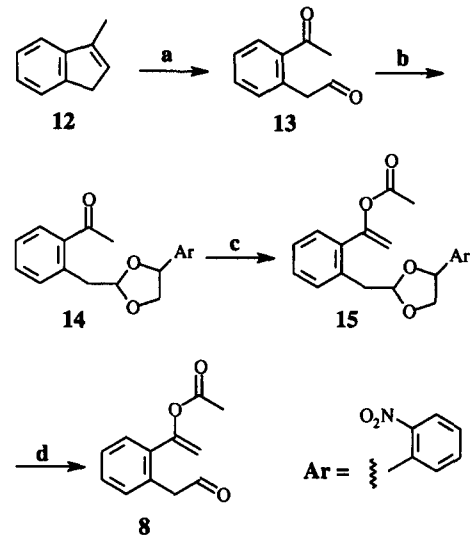

Figure 2. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C; Zn, HOAc, 0 °C (89%); (b) *ortho*-nitrophenylethylene glycol, *para*-$MeC_6H_4SO_3H$, benzene, reflux (52%); (c) KHMDS, DME, −78 °C; $CH_3C(O)Cl$, DMAP, −78 °C (52%); (d) *hv* (350 nm), benzene, rt (45%).

Results

Synthesis of substrate 8

A simple retrosynthetic analysis based on functional group interchange illustrated that the key intermediate for the synthesis of substrate **8** is ketoaldehyde **13**, which can be obtained by oxidative cleavage of the double bond in 1-methylindene (Fig. 2). Ozonolysis of **12** followed by reduction with zinc in acetic acid,[17] produced **13** in 89% yield, and in sufficient purity for direct use in the next reaction. To convert ketoaldehyde **13** to enol acetate **8**, first the aldehyde group must be protected. For our purpose, a proper protective group should possess three fundamental qualities: (1) efficiency and specificity for aldehyde group protection in the presence of a ketone; (2) stability under strongly basic conditions during the generation of enolate; and (3) specificity of removal. We tried several different kinds of carbonyl protective groups and removal conditions.[18] Some common protective groups can meet criteria 1 and 2, but the removal of these groups lacked the desired selectivity. For example, ethylene glycol efficiently and specifically protected the aldehyde group in the present of ketone and the resulting dioxolane acetal tolerated the strongly basic conditions used to form the enol acetate. Unfortunately, attempts at selective deprotection were unsuccessful. Transacetalization with acetone catalyzed by pyridinium tosylate (PPTS) gave the starting material back. Under acidic conditions, using either aq HCl solution or Lewis acids, bicyclic products were obtained.

We finally succeeded in the removal of the protective group by means of a photochemical reaction.[19] Selective protection of the ketoaldehyde **13** at the aldehyde carbonyl with *ortho*-nitrophenylethylene glycol pro-

duced acetal **14**. It should be noted that **14** was obtained as a mixture of diastereomers, since there are two chiral centers in this molecule. These isomers exhibited different R_f values on TLC and different proton NMR chemical shifts. Treatment of **14** with potassium bis(trimethylsilyl)amide in dimethoxyethane at −78 °C followed by acetylation with acetyl chloride gave enol acetate **15**, again as a mixture of stereoisomers. Deprotection of **14** by photolysis at 350 nm in benzene for 6 h afforded substrate **8** as an oily liquid in modest yield.

Hapten synthesis

Our initial synthetic approach to hapten **11** involved a two-component coupling synthesis in which an appropriate phosphonate side chain is installed on the cyclic phosphinate **20**. This compound has been previously synthesized via the route shown in Figure 3(A).[20,21] Unfortunately, we found that this reaction sequence is very capricious. One main problem is that the trivalent phosphorous intermediate **17** is very sensitive to moisture and air. Thus, the best yield we obtained of phosphinate **18** was only 17%, and frequently none of the desired compound was produced.

To avoid the formation of a trivalent phosphorous intermediate and thus improve the yield, an alternative route to **20** was investigated. As shown in Figure 3(B), the Arbuzov reaction[22] of benzyl bromide and triethyl phosphite gave the stable pentavalent phosphonate diester **22**. Conversion of **22** to phosphonochloridate **23** followed by a C–P coupling reaction[23] with the enolate of *tert*-butyl acetate gave **24** in an overall yield of 31%. Compared with de Graaf's route, this procedure has the advantages of stability of the intermediate **22** and increased reproducibility. But due in part to a competing O–C coupling reaction, the yield of C–P coupling product **24** is still relatively low.

The second component, phosphonic acid monoester **26**, was prepared in two steps. The Arbuzov reaction of methyl-5-bromovalerate and trimethylphosphite afforded phosphonate **25** in modest yield. Selective monodemethylation with *tert*-butylamine[24] gave the phosphonic acid monoester **26** (Fig. 4). With both components in hand, the next step was to attach the phosphonate side chain **26** to the cyclic phosphinate **20**. The synthesis of phosphonates from secondary alcohols is frequently a difficult transformation to undertake. Due to small amounts of intermediate **20** available, even with the improved route, we used commercially available 1-tetrahydronaphthol **27** for model investigations of the phosphonate coupling conditions. Campbell has developed a Mitsunobu coupling method for the synthesis of phosphonates from phosphonic acid monoesters and primary and secondary alcohols.[25] Malachowski and Coward have recently shown this to be the only effective method for the difficult synthesis of a complex secondary phosphonate.[26] But in our model investigation, this procedure did not lead to the coupling of **26** and **27**, despite a variety of attempts under different conditions. Since this reaction is

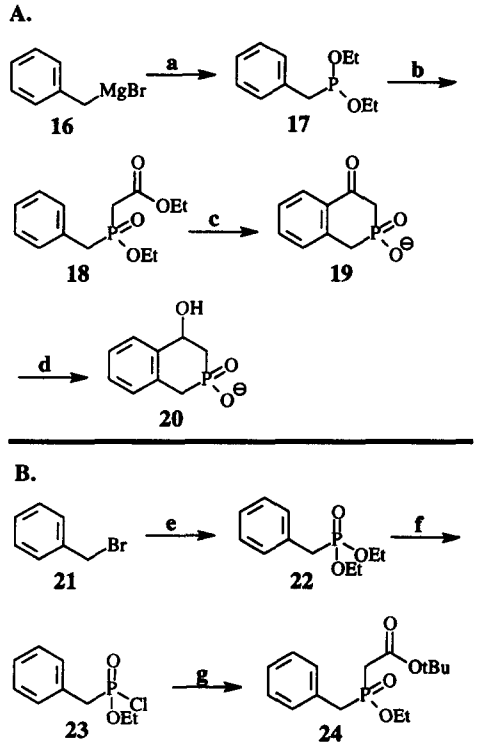

Figure 3. Reagents and conditions: (a) $P(OEt)_3$, Et_2O/benzene, rt; (b) $ClCH_2CO_2Et$, neat (17% from **16**); (c) (i) concd HCl, reflux; (ii) polyphosphoric acid, 130 °C; H_2O (17%); (d) $NaBH_4$, $CeCl_3{\cdot}6H_2O$, CH_3OH, rt (87%); (e) $P(OEt)_3$, xylene, reflux (80%); (f) PCl_5, benzene, reflux; (g) CH_3CO_2tBu, LDA, THF, −78 °C; **23**, −78 °C (31% from **22**).

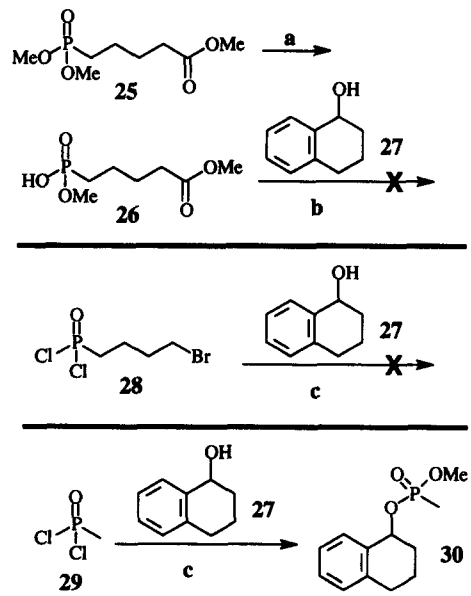

Figure 4. Reagents and conditions: (a) *tert*-$BuNH_2$, rt (~100%); (b) **27**, $iPrO_2CN{=}NCO_2iPr$, PPh_3, THF, rt; (c) **27**, Et_3N, 1H-tetrazole, benzene, 0 °C to rt; CH_3OH; (d) **27**, iPr_2NEt, 1H-tetrazole, benzene, 0 °C to rt; CH_3OH (48%).

sensitive to the steric effects of the alcohol component, the hindered secondary hydroxyl group in tetrahydronaphthol may account for the failure of the Mitsunobu condensation.

Another phosphonate synthesis method, developed recently by Zhao and Landry,[27a] involves the mono addition of alcohols to phosphonic dichlorides catalyzed by 1H-tetrazole. Quenching with methanol then results in the methyl-protected phosphonate. Zhao and coworkers have used this procedure to generate phosphonate derivatives of the complex secondary alcohols cholesterol[27a] and ecgonine methyl ester.[28] In our investigations, attempts to couple tetrahydronaphthol with the previously described long-chain alkyl-phosphonic dichloride **28** were unsuccessful,[27b] but switching to methyl phosphonic dichloride **29** gave the coupling product **30** in a moderate yield. Since the installation of a long phosphoryl side chain to the hindered hydroxyl group is difficult to achieve, and a short phosphoryl side chain can be coupled smoothly to the hydroxyl group, the alternative hapten **33** was designed. The proposed synthesis of the alternative hapten **33** is shown in Figure 5. The spacer group was first installed on the cyclic phosphinate **19** by coupling it with commercially available methyl-5-bromovalerate. Reduction with $NaBH_4$ gave the hydroxy compound **31**. However, attempts to couple methylphosphonic dichloride with **31** failed to produce **32**, giving instead the eliminated, thermodynamically stable compound **34**. Presumably, this elimination reaction is facilitated by the acidity of protons at the α-carbon of a phosphinate and the stability of the resulting conjugated double bond.[16,29] At this point in the synthesis, it was necessary to modify the aldolase hapten again to make it a synthetically stable compound. As depicted in Figure 6, the cyclic tetrahedral phosphinate group in **33** is replaced by a tetrahedral carbon to give the monofunctional phosphonate hapten **39**. The spacer group, which will be linked to a carrier protein, is on the nonessential 5-position of 1,2,3,4-tetrahydronaphthol. The negatively charged phosphonate group of **39**, which mimics the transition state of enol acetate hydrolysis, still remains as the primary determinant for immune recognition. This hapten design still should theoretically generate effective aldol catalysts, because enolate generation may be the rate-determining step in the aldol reaction. However, note the kinetic studies described in the Discussion section.[30]

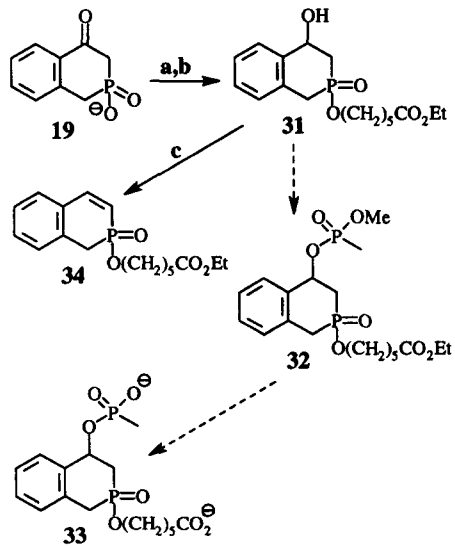

Figure 5. Reagents and conditions: (a) $Br(CH_2)_5CO_2Et$, KI, K_2CO_3, DMF ~70 °C, (30%); (b) $NaBH_4$, $CeCl_3 \cdot 6H_2O$, CH_3OH, rt (80%); (c) $CH_3P(O)Cl_2$, iPr_2NEt, 1H-tetrazole, THF, 0 °C to rt; CH_3OH.

The synthesis of **39** started from commercially available 5-hydroxytetralone (**35**). Alkylation with methyl-5-bromovalerate attached the linker arm to give compound **36**, which was reduced with $NaBH_4$ to give alcohol **37**. Coupling of **37** with methyl-phosphonic dichloride as described above, proceeded smoothly; however, deprotection to give **39** proved to be problematic. Initially we tried deprotection of the phosphonate moiety of **40** first, followed by hydrolysis of carboxylate methyl ester with NaOH (Fig. 7). This protocol failed; the P–O bond of phosphonate **40** was cleaved and alcohol **42** was produced. Hydrolysis of carboxylate methyl ester first, followed by deprotection of phosphonate with trimethylsilyl bromide,[31] gave the desired trimethylsilyl ester **44**. However, aqueous work up cleaved the P–O bond and also gave **42**. Finally, we solved the deprotection problem by simply quenching the coupling reaction with water to produce free phosphonic acid **38**, as shown in Figure 6. Hydrolysis of carboxylate methyl ester with lithium hydroxide in acetonitrile[32a] then gave the desired hapten **39**.[32b]

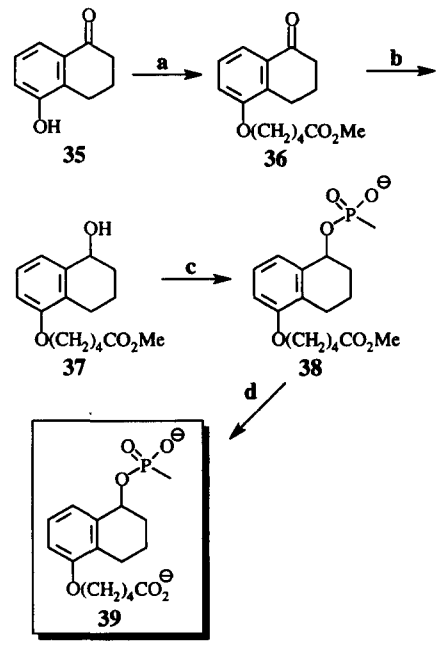

Figure 6. Reagents and conditions: (a) $Br(CH_2)_4CO_2Me$, K_2CO_3, DMF ~50 °C, (93%); (b) $NaBH_4$, $CeCl_3 \cdot 6H_2O$, CH_3OH, 0 °C to rt (80%); (c) $CH_3P(O)Cl_2$, iPr_2NEt, 1H-tetrazole, THF, 0 °C to rt; H_2O (61%); (d) 0.25 N LiOH, CH_3CN, rt (~100%).

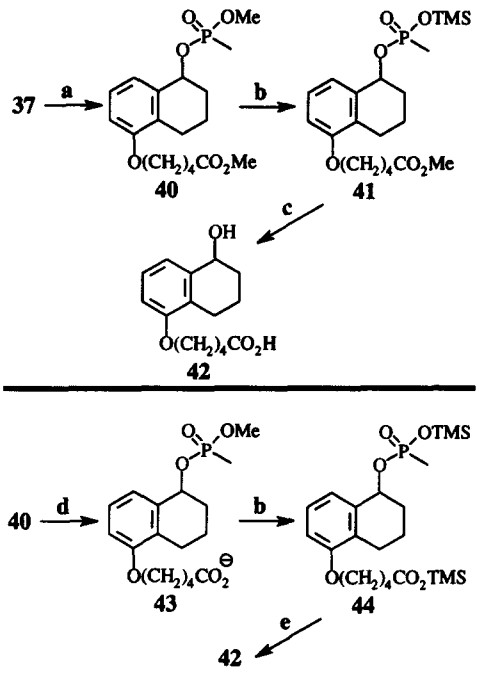

Figure 7. Reagents and conditions: (a) $CH_3P(O)Cl_2$, iPr_2NEt, 1H-tetrazole, THF, 0 °C to rt; CH_3OH (75%); (b) Me_3SiBr, CH_2Cl_2, rt; (c) 0.5 N NaOH, CH_3CN; (d) 0.25 N LiOH, *tert*-$BuNH_2$, rt (68%); (e) dilute aq HCl.

The direct and efficient synthetic route to **39** provided us with an opportunity to prepare it asymmetrically. We proposed that an enantiomerically enriched version of hapten **39** could induce antibodies that would catalyze the aldol reaction to give product **10** with a defined stereochemical outcome. We therefore used asymmetric reduction of **36** followed by coupling and deprotection as described above to produce the enantioenriched hapten (Fig. 8). The oxazaborolidine-catalyzed borane reduction procedure developed by Corey and Link was used for this reduction.[33] The catalyst **45** was prepared following the procedure of the Merck group.[34] The chiral alcohol **46** was obtained in 93% yield with this catalyst.

According to the reaction mechanism, this asymmetric reaction should afford alcohol **46** with the *R* configuration. Both *R*-tetrahydronaphthol and **46** show negative optical rotation, and this provides evidence that the absolute configuration of alcohol **46** is *R*. Conformation was provided by the fact that the same chiral catalyst used for the production of **46** reduces tetrahydronaphth-1-one to *R*-tetrahydronaphth-1-ol. To determine the enantiomeric purity of **46**, it was converted to the Mosher's ester derivative as described in the Experimental section. Integration of proton NMR signals from the two diastereomers indicates that **46** was obtained in 95% enantiomeric excess.

Under the same conditions that were used to make racemic hapten **39**, the (*R*)-alcohol **46** was coupled smoothly with methylphosphonic dichloride to produce **47** in 62% yield. Efficient hydrolysis of the methyl ester cleanly provided the enantiomerically enriched hapten

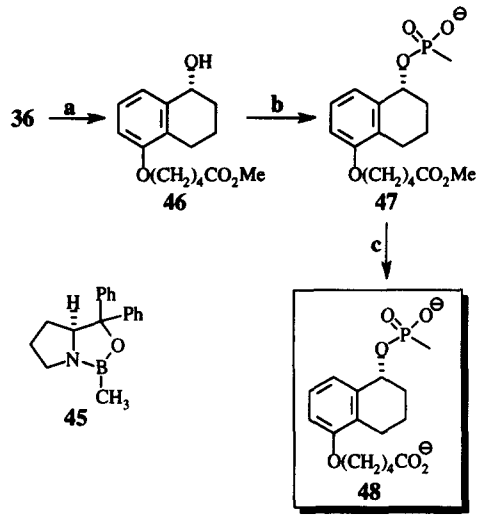

Figure 8. Reagents and conditions: (a) **45**, $BH_3.Me_2S$, THF, approximately −15 °C (93%); (b) $CH_3P(O)Cl_2$, iPr_2NEt, 1H-tetrazole, THF, 0 °C to rt; H_2O (62%); (c) 0.25 N LiOH, CH_3CN, rt (~100%).

48. Neither the coupling nor the deprotection reaction should affect the chiral center.

Induction and characterization of polyclonal antibodies

The majority of studies on catalytic antibodies have utilized monoclonal antibodies. Monoclonal antibodies obtained via hybridoma technology are homogeneous; that is, each antibody molecule has an identical amino acid sequence and the same binding and catalytic properties. Therefore, the characterization of individual antibodies is meaningful and reproducible. Polyclonal antibodies, on the other hand, are heterogeneous mixtures of IgG antibody species formed from a large number of antibody-producing cells. This inherent heterogeneous nature and the variability of the polyclonal response between individual animals are significant disadvantages. However, compared with monoclonal antibodies, using polyclonal antibodies also has certain advantages. First, polyclonal antibodies can be isolated directly from the serum of immunized animals using protein G chromatography. The time-scale for preparing polyclonal antibodies is thus much shorter than that of monoclonal antibodies. In addition, the techniques required to isolate polyclonal antibodies are much easier and do not require any tissue culture manipulations. Furthermore, the costs for making polyclonal antibodies are dramatically less than that of monoclonal antibodies.

Thus, for the preliminary evaluation of new hapten designs, the polyclonal protocols can provide important preliminary data and these can be used to justify further investigations. Gallacher et al.[35] and Wilmore and Iverson[36] have described carbonate and ester hydrolysis reactions catalyzed by polyclonal antibodies, in which the range of apparent catalytic rate enhancement (k_{cat}/k_{uncat}) is 500–1800. We therefore prepared polyclonal antibodies for our characterization of haptens **39** and **48**. The haptens were coupled to the carrier protein

keyhole limpet hemocyanin (KLH) using EDC as described in the Experimental section. The hapten–KLH conjugates were used to immunize New Zealand white rabbits. After three booster immunizations, the serum of the rabbits was separated and the IgG immunoglobulins were isolated from the other serum proteins using protein G affinity chromatography. The pure IgG fraction was then concentrated and dialyzed into tris buffer (pH 8.4, 25 mM). The final concentration of antibody solution was adjusted to 25 μM with Tris buffer for the catalytic assays.

The first step in our model reaction is the hydrolysis of enol acetate **8** to give reactive intermediate enolate **9**, which then undergoes an intramolecular aldol reaction to form the new carbon–carbon bond. The whole reaction sequence is 'triggered' by the hydrolysis of enol acetate **8**. Therefore, we examined the ability of the polyclonal antibodies to catalyze the hydrolysis of an ester bond. The tetrahydronaphthyl acetate **49** (Fig. 9) was used as a substrate to assay the catalytic ability of polyclonal antibodies, and the reaction was analyzed by HPLC as described in the Experimental section.

We first characterized antibodies from rabbits A1 and A2, which were immunized with the hapten **39**–KLH conjugate. Polyclonal antibody preparation A1 did not accelerate the hydrolysis of ester **49**, compared to a control reaction. However, in the presence of 25 μM polyclonal antibody A2 the hydrolysis of ester **49** was ~40% faster than the background reaction, and this acceleration was completely inhibited by 100 μM of hapten **39**. This rate acceleration was not seen with purified preimmunization polyclonal antibodies from the same animal or with polyclonal antibodies induced by a different antibody. This hydrolytic activity was not characterized further.

Since the polyclonal antibody A2 appeared to accelerate the hydrolysis of ester **49**, it was then presumed that it would also be capable of catalyzing the intramolecular aldol condensation as we proposed before. To test this idea, enol ester **8** was used as substrate and assays were performed under the same conditions described above. In both the presence and the absence of the polyclonal antibody A2, products **10**, **13**, and **51** were observed (Fig. 9). The relative amounts of ketoaldehyde **13**, bicyclic compound **10**, and naphthol **51** did not show regular changes; the relative amounts of these products varied from reaction to reaction. The rapid spontaneous reaction of **8** to give **13**, and of **10** to give **51**, interfered with the accurate determination of the rate of acceleration for the aldol condensation. We were not able to determine whether the formation of **13** is due to the protonation of enolate **9** in the antibody binding pocket or protonation in the reaction solution.

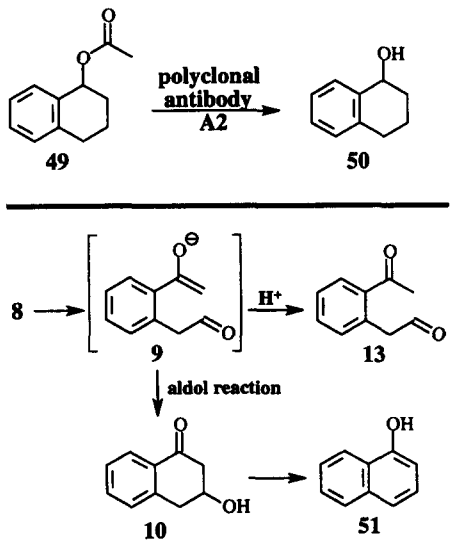

Figure 9.

Polyclonal antibody preparation B was generated from a rabbit immunized with a KLH conjugate of the enantiomerically enriched hapten **48**. We hypothesized that these antibodies would discriminate between the different enantiomeric forms of **49** in the ester hydrolysis reaction. Enantioselective esterolytic antibodies have been previously induced by chiral phosphonate haptens.[37] However, antibody B did not show any catalytic activity with the *R* isomer of **49**, the *S*-ester, or the racemic substrate. Preliminary experiments also demonstrated that it could not catalyze the cyclization of **8** to give the aldol product **10**.

Discussion

Studies of the model phosphonate coupling of tetrahydronapth-1-ol have provided further evidence of the utility of the selective phosphonic dichloride coupling procedure developed by Landry and co-workers, particularly for the synthesis of hindered secondary phosphonates. We have also described a modification of this procedure, in that quenching with water rather than a second alcohol leads directly to the unprotected phosphonate. This modification may prove to be useful for other schemes where the free phosphonate is generated in the ultimate or penultimate synthetic step.

One of the polyclonal preparations studied appeared to accelerate the hydrolysis of the secondary acetate **49**. However, none of the polyclonal antibodies appeared to accelerate the desired aldol condensation. There are several possible reasons for the lack of aldolase activity. The heterogeneous nature of polyclonal antibodies may prevent the detection of modestly efficient aldolase catalysts that are present in small amounts. The lack of congruity between the hapten **39** and substrate **8** may hinder its binding to the induced antibodies, although this has been overcome in certain cases, such as the aldol cyclization of **1** to **2**. Alternatively, the aromatic and hydrophobic nature of the product **10** may lead to its tight binding to the antibody, and thus prevent turnover.

The observed lack of catalytic activity may also result from the monofunctional nature of hapten **39** and the mechanism of the anionic aldol reaction. The success of our proposed aldolase pathway depends on the cyclization of enolate intermediate **9** to **10** occurring faster than its protonation to **13** (Fig. 9). Nagorski and co-workers have performed detailed kinetic studies on the closely related aldol reaction of **52** (Fig. 10).[30] They have found that the cyclization of enolate **53** is indeed faster than its protonation by water (k_{H_2O}). However, its protonation by ammonium cations (k_{R_3NH}) is significantly faster than cyclization. Thus if a lysine or arginine side chain is present in the antibody combining site it may protonate **9** before it cyclizes. Richard proposed that carbonyl addition will be rate-limiting for aldolases, unless the carbonyl group is activated in some way in the enzyme active site. However, activation of the carbonyl group alone is not enough to catalyze the aldol reaction. Flanagan and co-workers have characterized antibodies induced by the phosphinate hapten **58**.[9] The phosphinate moiety presumably induces a pocket that can help stabilize the attack of the enolate of **55** on the benzaldehyde carbonyl. Although one of these antibodies catalyzed a retro-aldol reaction, it was not able to accelerate the aldol condensation of **55** and **56** to give **57**. Thus it appears that bifunctional haptens, which induce antibodies that both accelerate enolization and attack of the enolate on the carbonyl, will be necessary for the development of efficient aldolase antibodies of the type proposed in this paper.[38]

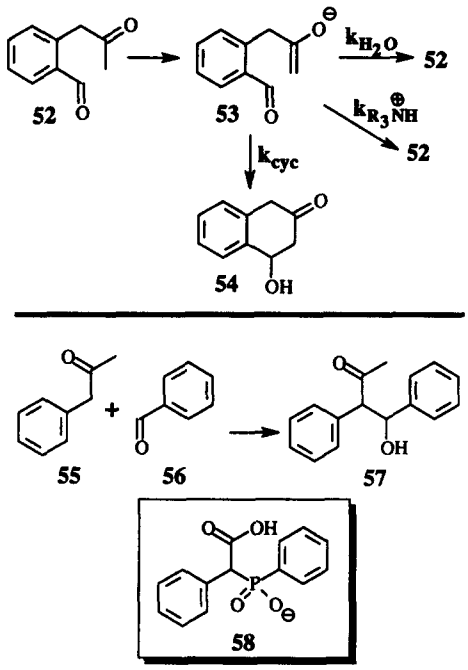

Figure 10.

Experimental

General procedures

All moisture/air-sensitive reactions were conducted in flame or oven-dried glassware under an dry argon atmosphere. Moisture-sensitive reagents were transferred by syringe and introduced to the reaction vessel through rubber septum caps. All reaction contents were stirred magnetically. Solvents, and organic and inorganic reagents were obtained from the following suppliers: Aldrich Chemical Company, Fisher Scientific, and Janssen Chimica. Unless otherwise stated, all reagents were used without further purification. The following solvents were prepared anhydrously prior to use as described: THF, DME, and diethyl ether were distilled from Na/benzophenone; CH_2Cl_2 was distilled from CaH_2; methanol was distilled from Mg; benzene was distilled from Na. Toluene and DMF were dried by storage over activated 3-Å molecular sieves.

Flash column chromatography was performed with silica gel 60 (230–440 mesh, E. Merck). Thin-layer chromatography was conducted on precoated alumina plates (silica gel 60 F-254, Merck), and was visualized by ultraviolet illumination or by spraying with 8% phosphomolybdic acid (PMA) in ethanol, followed by heating. Reversed-phase HPLC was conducted on a Waters system with a U6K injector, two 501 pumps, and a 490E multiwavelength UV detector.

Optical rotations were measured with a Perkin–Elmer Model 241 MC polarimeter. Melting points were taken on a MEL-TEMP II melting point apparatus using a FLUKE 51-K/J thermometer and are uncorrected. Nuclear magnetic resonance spectra were recorded at either 300 MHz (^{1}H NMR) or 76.5 MHz (^{13}C NMR) on a General Electric QE-300 spectrometer, with the exception of ^{31}P spectra, which were recorded at 121 MHz on a GN-300 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) using an internal TMS standard. Multiplicities are indicated by the following symbols: (br) broad, (ap) apparent, (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded on a Kratos MS 80 RFA mass spectrometer at 70 eV.

1-Methyl-indene 12. 1-Indanone (1.0 g, 7.4 mmol) in 3 mL of THF at 0 °C under an argon atmosphere was added to a solution of methyl magnesium chloride (3.0 M in THF, 3.8 mL, 11.4 mmol). The reaction mixture was stirred at 0 °C for 2.5 h and quenched with 20 mL of 10% aq HCl. The solution was extracted with ether (3 × 25 mL), washed with satd aq NaCl and dried over anhyd $MgSO_4$. The crude product was purified by flash chromatography (9:1 hexanes:ethyl acetate). The product 1-methylindan-1-ol was obtained as a pale yellow solid (0.8 g, 73%). ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 3H, CH_3), 2.22 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 7.24 (m, 3H, ArH), 7.36 (m, 1H, ArH). A solution of 1-methylindan-1-ol (700 mg, 4.73 mmol) and 3 mg of *para*-toluenesulfonic acid in 50 mL of benzene was refluxed under argon for 1 h. The solvent was removed using a rotary evaporator and the residue was purified on a flash column (hexanes). The product was obtained

as a colorless liquid (580 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H, CH_3), 3.33 (s, 2H, CH_2), 6.21 (s, 1H, CH), 7.34 (m, 4H, ArH).

***o*-Acetophenyl-acetaldehyde 13**. A solution of **12** (4.5 g, 34.6 mmol) in 20 mL of CH_2Cl_2 was cooled to −78 °C and ozone (flow rate 1 L/min) was bubbled through for 1.5 h. The resulting green solution was flushed with N_2 until the green color disappeared. The ozonide was quenched with 3 g of zinc dust and 20 mL of acetic acid and stirred at 0 °C for 3 h. The resulting mixture was then filtered, the filtrate was washed with 5% aq K_2CO_3 (2 × 30 mL) and satd NaCl soln (2 × 50 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, 50 mL of dry benzene was added, and the mixture was heated to remove the benzene by rotary evaporation. The residue was pumped at high vacuum for 2 h. Five grams (89%) of the product were obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.62 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.25–7.87 (m, 4H, ArH), 9.78 (s, H, CHO).

(*ortho*-2-(*ortho*-Nitrophenylethylenedioxy)ethylene)phenyl-acetone 14. A mixture of **13** (0.5 g, 3.1 mmol), *ortho*-nitrophenylethylene glycol (1.7 g, 9.3 mmol), and *para*-toluenesulfonic acid (20 mg) in anhyd benzene (80 mL) was added to a flask which was equipped with a Dean–Stark trap. The flask, trap, and condenser were protected from daylight with aluminum foil. The solution was refluxed under argon for 2 h and cooled to room temperature. Excess *o*-nitrophenylethylene-glycol (1.0 g) was recovered by filtration. The filtrate was concentrated and the residue was purified by flash chromatography (8:2 hexanes:ethyl acetate). After solvent removal, acetal **14** (527 mg, 52%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.57 (2H, CH_2), 3.81 (q, 1H) 4.42 (t, 1H, CH), 5.25 (t, 1H, CH) 5.20 (m, 1H), 7.36–8.07 (m, 8H, ArH).

(*ortho*-2-(*ortho*-Nitrophenylethylenedioxy)ethylene)acetyl-styrene 15. Acetal **14** (0.5 g, 1.53 mmol) in 5 mL of dimethoxyethane (DME) was added to a solution of KHMDS (potassium bis(trimethylsilyl)amide; 0.5 M in toluene, 4.6 mL, 2.3 mmol) at −78 °C under argon, and the reaction was stirred at −78 °C for 15 min. The resulting enolate solution was then added to a solution of acetyl chloride (0.22 mL, 3.06 mmol) in 5 mL of DME containing 9.4 mg of *N,N*-dimethylaminopyridine (DMAP). After 20 min at −78 °C, the reaction mixture was warmed to room temperature. Hexane (30 mL) was added to the reaction mixture, and the resulting solution was washed with satd NaCl aq soln (2 × 15 mL). The organic layer was dried over anhyd $MgSO_4$, and concentrated by rotovap. The residue was purified by flash chromatography (8:2 hexanes:ethyl acetate) and enol acetate **15** was obtained as a yellow oil (250 mg, 52%), and 72 mg of starting material were also recovered. ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H, CH_3), 3.41 (d, 2H, CH_2), 3.86 (q, 1H, CH), 4.45 (t, 1H), 5.13 (s, 1H, vinylic H), 5.23 (s, 1H, vinylic H), 5.30 (m, 1H), 5.56 (m, 1H), 7.32–8.06 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$) δ 21.12 (CH_3), 36.35 (CH_2), 72.32 (OCH), 74.07 (CH_2O), 76.00 (CH), 105.02 (=CH_2), 106.64 (=C), 124.59–137.72 (12 ArC), 153.44 (–C=O).

(*ortho*-Acetaldehyde)acetyloxy-styrene 8. A solution of **15** (0.1 g, 0.27 mmol) in 80 mL of anhyd benzene was irradiated at 350 nm under an N_2 atmosphere for 6 h. Solvent was removed by rotary evaporator and the residue was purified on a flash column (8:2 hexane:ethyl acetate). After the removal of the solvent, aldehyde **8** was obtained as a yellow oil (25 mg, 45%). ^{1}H NMR ($CDCl_3$) δ 2.01 (s, CH_3), 3.88 (d, J = 1.5 Hz, CH_2), 5.01 (d, J = 1.5 Hz, 1H, vinylic H), 5.20 (d, J = 1.5 Hz, 1H, vinylic H), 7.21–7.46 (m, 4H, ArH), 9.77 (t, J = 1.5 Hz, CHO). ^{13}C NMR 48.45, 106.83, 119.71, 127.45, 129.34, 129.99, 130.54, 130.79, 168.81, 199.66.

Diethyl-benzylphosphonate 22. Benzyl bromide (**21**, 7.23 g, 42 mmol) and triethyl phosphite (6.38 g, 38 mmol) were dissolved in xylene (50 mL) and the resulting solution was refluxed overnight. The solvent was removed (high vacuum rotovap) and distillation of the residue afforded 6.9 g (80%; bp: 92–94 °C, ~0.05 mm) of the desired phosphonate **22** as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6H, CH_3), 3.17 (d, 2H, $C\underline{H}_2Ph$), 4.02 (m, 4H, CH_2), 7.31 (narrow m, 5H, $C_6\underline{H}_5$). ^{31}P NMR (121 MHz) δ 26.75.

***tert*-Butyl-3-ethoxy-(3-oxo-3-phospha)-4-phenylbutanoate 24**. A solution of phosphonate **22** (3.0 g, 13.1 mmol) and PCl_5 (3.01 g, 14.5 mmol) in benzene (15 mL) was refluxed under argon for 3 h. The solvent was then removed to give the crude phosphonochloridate **23**: ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, CH_3), 3.53 (d, 2H, $C\underline{H}_2Ph$), 4.27 (m, 2H, CH_2), 7.34 (app s, 5H, C_6H_5).

In a separate flask, a solution of LDA was prepared by adding *n*-BuLi (1.6 M in hexanes, 9.8 mL, 15.7 mmol) to diisopropylamine (2.29 mL 16.4 mmol) in THF (5.0 mL) at −78 °C. After 15 min, the LDA solution was added dropwise to a mixture of *tert*-butyl acetate (2.12 mL, 15.7 mmol) and THF (5.0 mL), also at −78 °C. After an additional 30 min at −78 °C, a solution of the above crude phosphonochloridate **23** in THF (20 mL) was added to the enolate reaction mixture over 10 min. The reaction mixture was stirred for 1 h at −78 °C, 1 h at 0 °C, diluted with ether, and then quenched with 10% aq HCl. The ether layer was washed with H_2O, satd $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatographic purification (1:1 ethyl acetate: hexanes) afforded 1.2 g (31%) of the desired phosphinate **24**. ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, CH_3), 1.51 (s, 9H, *t*-Bu), 2.80 (dd, 2H), 3.34 (m, 2H), 4.09 (m, 2H, OCH_2), 7.3 (m, 5H, C_6H_5).

5-(Methyl-5′-oxyvalerate)-1-tetralone 36. A mixture of 5-hydroxy-1-tetralone (**35**, 100 mg, 0.62 mmol), K_2CO_3 (128 mg, 0.92 mmol) and methyl 5-bromovalerate in 3 mL of DMF was stirred at 50 °C for 2 h. The reaction mixture was taken up with 20 mL of ethyl acetate and washed with 10 mL satd NH_4Cl and 10 mL of satd NaCl soln. The organic layer was dried over anhyd $MgSO_4$, filtered, and concentrated. After purification by flash

chromatography (4:1 ethyl acetate:hexanes), 6 mg of **35** was recovered and 149 mg of product was obtained as a white solid (93%, based on consumed starting material: mp 72–74 °C). ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 4H, H3′ and H4′), 1.12 (m, 2H, H3), 2.43 (t, 2H, H2′), 2.65 (t, 2H, H4), 2.91 (t, 2H, H2), 3.69 (s, 3H, CH_3O), 4.01 (t, 2H, H5′), 6.99 (d, 1H, H6), 7.25 (t, 1H, H7), 7.64 (d, 1H, H8). ^{13}C NMR ($CDCl_3$) δ 21.70 (C3), 22.51 (C3′), 22.84 (C4), 28.69 (C4′), 33.64 (C2′), 38.85 (C2), 51.59 (CH_3O), 67.73 (C5′), 115.05 (C5), 118.69 (C7, C9), 126.66 (C6), 133.65 (C10), 156.06 (C8), 173.85 (C1′), 198.75 (C1).

5-(Methyl-5′-oxypentanoate)-1,2,3,4-tetrahydro-1-naphthol 37. Compound **36** (140 mg, 0.51 mmol) and $CeCl_3 \cdot 6H_2O$ (285 mg, 0.77 mmol) were dissolved in 5 mL of methanol and the resulting solution was cooled to 0 °C. Sodium borohydride (38.6 mg, 1.02 mmol) was added to the solution in portions and the reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated, the residue was taken up with 20 mL of ethyl acetate, washed with satd NaCl and dried over $MgSO_4$. The solvent was removed by rotary evaporation and the crude product was purified by flash chromatography (4:1 hexanes:ethyl acetate). Pure **37** (134 mg, 95%) was obtained as a white solid (mp 68 °C). ^{1}H NMR ($CDCl_3$) δ 1.66 (d, 1H, OH), 1.77–1.97 (m, 8H, H3, 4, 3′, and 4′), 2.41 (t, 2H, H2′), 2.64 (m, 2H, H2), 3.68 (s, 3H, CH_3O), 3.97 (t, 2H, H5′), 4.76 (m, 1H, H1), 6.72 (d, 1H, H8), 7.05 (d, 1H, H6), 7.15 (t, 1H, H7). ^{13}C NMR ($CDCl_3$) δ 18.02 (C3), 21.77 (C3′), 23.03 (C4), 28.76 (C4′), 31.69 (C2), 33.67 (C2′), 51.56 (CH_3O), 67.21 (C5′), 68.18 (C1), 109.42 (C5), 120.42 (C9,C7), 126.47 (C6), 140.02 (C8), 156.29 (C10), 173.92 (C1′).

5-(Methyl-5′-oxypentanoate)-1,2,3,4-tetrahydro-1-naphthyl-methylphosphonate 38. A solution of **37** (83 mg, 0.23 mmol), 1-H tetrazole (2 mg, 0.023 mmol) and diisopropylethyl amine (0.33 mL, 1.87 mmol) in 4 mL of THF was stirred under argon at room temperature for 15 min. The solution was then cooled to 0 °C, methylphosphonic dichloride (93.5 mg, 0.70 mmol) in 1.0 mL of THF was added dropwise and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by adding 0.2 mL of H_2O at 0 °C and stirring was continued for 15 min. The solvent was evaporated, the residue was diluted with 2 mL of H_2O, and washed with hexane (2 × 5 mL). The hexane layers were combined, dried over $MgSO_4$, filtered, and concentrated, to give 42 mg of recovered **37**. The aqueous solution was adjusted to pH 2 with 10% HCl and extracted with ethyl acetate (4 × 5 mL). The organic layers were combined, dried over $MgSO_4$ and evaporated to give 21 mg of **38** as an oil (61%, based on consumed starting material). An analytical sample of **38** was obtained by reversed-phase HPLC using a program of 5 min of 100% A followed by a linear gradient of 100% A to 100% B over 30 min (A: 25 mM aq NH_4HCO_3 (pH 8.0); B: CH_3CN; Vydac pH-stable C_8 10 × 250 mm column; flow rate: 3.0 mL; UV monitoring at 214 and 254 nm). The retention time of the phosphonate **38** under these conditions was 16 min, and the identity of this peak was confirmed by proton NMR of the isolated analytical sample. ^{1}H NMR (MeOH-d_4) δ 1.32 (d, 3H, $^1J_{HP}$ = 16.5 Hz, CH_3), 1.82–2.05 (m, 8H; H3, 4, 3′, and 4′), 2.42 (t, 2H, H2′), 2.62 (m, 2H, H2), 3.65 (s, 3H, OCH_3), 3.97 (t, 2H, H5′), 5.28 (m, 1H, H1), 6.76 (d, 1H, H8), 7.11 (m, 2H, H6, 7).

5-(5′-Oxypentanoic acid)-1,2,3,4-tetrahydro-1-naphthylmethylphosphonate 39. To the solution of **38** (21 mg, 0.06 mmol) in 1 mL of acetonitrile was added 0.25 N LiOH (0.35 mL, 0.09 mmol). The reaction was stirred at room temperature for 90 min. Solvent was evaporated and the residue was neutralized with 3% (v/v) acetic acid in ethyl acetate. After concentration by rotary evaporation (water aspirator and high vacuum), the product **39** was obtained as an oil. The purity of **39** was confirmed by reversed-phase HPLC using a program of 5 min of 100% A followed by a linear gradient of 100% A to 100% B over 30 min (A: 0.02% trifluoroacetic acid in H_2O; B: CH_3CN; Vydac pH-stable C_8 10 × 250 mm columm; flow rate: 3.0 mL; UV monitoring at 220 and 254 nm). The retention time of the hapten **39** under these conditions was 13 min. Using the HPLC conditions described above for **38** (with NH_4HCO_3/H_2O rather than TFA/H_2O as the aqueous solvent), **39** eluted at 5 min, shortly after the solvent front. ^{1}H NMR (MeOH-d_4) δ 1.32 (d, 3H, $^1J_{HP}$ = 16.2 Hz, CH_3), 1.76–2.10 (m, 8H, H3,4, 3′,4′), 2.36 (t, 2H, H2′), 2.64 (m, 2H, H2), 3.97 (t, 2H, H5′), 5.26 (m, 1H, H1), 6.78 (d, 1H, H8), 7.21 (m, 2H, H6, 7). 8.54 (b, 1H, OH). ^{13}C NMR (MeOH-d_4) δ 12.23 (d, $^1J_{PC}$ = 138 Hz, CH_3), 18.02 (C3), 21.49 (C4), 22.29 (C3′), 28.78 (C4′), 30.62 (C2′), 35.38 (C2), 67.21 (C5′), 70.46 (C1, d, $^2J_{PC}$ = 5.1 Hz), 109.10 (C5), 120.94 (C7), 125.85 (C6), 125.60 (C9), 138.11 (C8), 156.13 (C10), 177.08 (C1′). ^{31}P NMR (121 MHz) 23.50 ppm.

(*R*)-5-(methyl-5′-oxypentanoate)-1,2,3,4-tetrahydro-1-naphthol 46. To a solution of ketone **36** (130 mg, 0.47 mmol) in 5 mL of THF was added oxaborazole catalyst **45** (1.41 mL of a 1.0 M solution in toluene, 0.3 equiv; prepared as described by the Merck group[34]). The resulting solution was cooled to −15 °C and borane–methyl sulfide (33 μL, 0.33 mmol, dissolved in 0.5 mL of THF) was added dropwise under argon. Stirring was continued for 30 min at −15 to −10 °C. The reaction was quenched by the addition of 5 mL of methanol at −15 °C and stirred at room temperature for 30 min. The solvent and residual $BH_3 \cdot Me_2S$ were removed with a rotary evaporator at reduced pressure. The residue was purified by flash chromatography (4:1 hexanes:ethyl acetate), and 121 mg (93%) of product **46** was obtained as a white solid (mp 73–75 °C). The ^{1}H and ^{13}C NMR were identical to the racemic alcohol **37**.

Enantiomeric purity determination. A solution of **46** (15 mg, 0.054 mmol), (*S*)-α-methoxyphenylacetic acid (10 mg, 0.06 mmol), EDC (12.4 mg, 0.065 mmol) and DMAP (1 mg) in 2 mL of dichloromethene was stirred under argon at room temperature overnight. The solvent was evaporated and the residue purified by

flash chromatography (4:1 hexanes:ethyl acetate). The desired product was obtained as an oil (17.4 mg, 76%). Integration of the proton NMR signals for the aromatic H6 proton from the two diastereomers indicates a 95% excess of the major (*S*,*R*) ester.

(*R*)-5-(methyl-5′-oxypentanoate)-1,2,3,4-tetrahydro-1-naphthyl-methyl-phosphonate 47. A solution of **46** (50 mg, 0.18 mmol), 1H-tetrazole (2 mg), and diisopropylethylamine (0.25 mL, 1.44 mmol) in 4 mL of THF was stirred at room temperature for 15 min. The solution was then cooled to 0 °C, methylphosphonic dichloride (72 mg, 0.54 mmol) in 1 mL of THF was added dropwise, and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by adding 0.2 mL of water at 0 °C and stirring was continued for 15 min at room temperature. The solvent was removed and the residue was diluted with 2 mL of water and washed with hexane (2 × 5 mL). The aqueous solution was adjusted to pH 2 with 10% HCl and extracted with ethyl acetate (4 × 5 mL). The ethyl acetate layers were combined, dried over $MgSO_4$ and evaporated to give 40 mg (62%) of product **47**. ^{1}H NMR (MeOH-d_4) δ 1.31 (d, 3H, $^1J_{PH}$ = 16.5 Hz, CH_3), 1.82 (m, 6H, H3, 3, 4′), 2.01 (m, 2H, H4), 2.42 (t, 2H, H2′), 2.64 (m, 2H, H2), 3.65 (s, 3H, OCH_3), 3.97 (t, 2H, H5′), 5.28 (m, 1H, H1), 6.76 (d, 1H, H6), 7.12 (m, 2H, H6, 7).

(*R*)-5-(5′-oxyvaleric acid)-1,2,3,4-tetrahydro-1-naphthyl-methylphosphonate, dilithium salt 48. A 0.37 mL quantity of 0.25 N LiOH (0.09 mmol) at room temperature was added to a solution of **47** (25 mg, 0.07 mmol) in 1 mL of acetonitrile. The reaction was stirred for 90 min. The solvent was evaporated, and the product was obtained as a white solid (lithium salt). ^{1}H NMR (MeOH-d_4) δ 1.32 (d, 3H, $^1J_{PH}$ = 16.5 Hz, CH_3), 1.82 (m, 6H, H3, 3′, 4′), 2.01 (m, 2H, H4), 2.24 (t, 2H, H2′), 2.65 (m, 2H, H2), 3.98 (t, 2H, H5), 5.27 (m, 1H, H1), 6.77 (d, 1H, H8), 7.12 (m, 2H, H6, 7). ^{13}C NMR (MeOH-d_4) δ 12.78 (d, $^1J_{PC}$ = 140 Hz, CH_3), 18.05 (C3), 22.58 (C4), 23.00 (C3′), 29.10 (C4′), 30.65 (C2′), 37.46 (C2), 67.35 (C5′), 70.45 (C1, $^2J_{PC}$ = 5.1 Hz), 109.07 (C5), 120.87 (C7), 125.56 (C6), 125.85 (C9), 138.12 (C8), 156.16 (C10), 181.33 (C1′). ^{31}P NMR 23.37.

Hapten–KLH conjugate. Hapten **40** (7.5 mg, 0.021 mmol), (±)-1-hydroxy-2,5-dioxo-3-pyrrolidinesulfonic acid (monosodium salt hydrate, 5.0 mg, 0.023 mmol), and EDC (4.8 mg, 0.025 mmol) were added to a solution of 0.5 mL H_2O and 0.5 mL DMF, and the mixture was stirred at room temperature for 2.5 h. KLH (keyhole limpet hemocyanin, 0.6 mL, Sigma, reconstituted to 10 mg/mL with doubly distilled water) was added to 3.4 mL of potassium phosphate buffer (50 mM, pH 7.07), and 500 μL of the hapten–sulfoNHS solution was added to the protein solution. The resulting clear hapten–KLH conjugate solution was shaken at 4 °C for 24 h and stored at that temperature before use. The hapten **48**–KLH conjugate was prepared in the same manner.

Immunizations. All animal procedures were performed by WSU Department of Laboratory Animal Research staff. For immunizations, 5-lb male New Zealand white rabbits were used. In each injection, a mixture of 0.5 mL hapten–KLH conjugate solution and an equal volume of Freund's adjuvant (Sigma) was injected. Subsequent injections were made at 21-day intervals with incomplete Freund's adjuvant, and 10 mL of whole blood was removed from each bleed. The whole blood sample was separated into serum and cells by contrifugation at 10,000 *g* for 10 min. The resulting serum samples were stored at frozen at −30 °C.

Polyclonal IgG purification. The protein G-sepharose affinity column (1-mL column, 2-mg recombinant protein G/mL, part of the Pharmacia Mab TrapG-II kit which includes buffer solutions) was first washed with 8 mL of binding buffer before use. The serum sample was thawed, and 1 mL of crude serum was loaded onto the column. The column was first washed with 10 mL of binding buffer, followed by 5 mL of elution buffer. Fractions of 1 mL were collected and 10 μL of neutralizing buffer were added to each fraction. Each fraction was assayed for its ultraviolet absorbance at 280 nm, and the concentration of IgG was determined by assuming an extinction coefficient of 2.0×10^5. The pure IgG solution was then concentrated and dialyzed using a Microcon dialysis apparatus in a microcentrifuge (Millipore Ultrafree-MC; 30,000 NMWL, 4.3×10^3/min) vs Tris buffer (pH 8.4, 25 mM).

HPLC catalytic assay. The catalytic assays were conducted with a 100-μL total volume of reaction solution containing: polyclonal IgG (25 μM), substrate (1 mM), and tris buffer (pH 8.4, 25 mM). The control experiment was carried out in the absence of antibody under identical conditions. Reactions were incubated from 0 to 8 h at 37 °C. At every 1-h interval, 10-μL aliquots was removed and quenched with an equal volume of acetonitrile. The mixture was separated by reversed-phase HPLC (Vydac 4.6 × 250 mm C_8 column). A linear gradient of 100% H_2O to 100% CH_3CN over 30 min was used. The substrates and products were detected by UV at 225 nm and 254 nm. The retention time for each compound is as follows: substrate **8** 7.5 min, cyclic compound **10** 12 min, tetrahydronaphthol **50** 16 min, naphthol **51** 21 min, ketoaldehyde **13** 22.5 min, tetrahydronaphthyl acetate **49** 23 min. The concentrations were determined from peak integrations calibrated with standard curves.

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