of the N-Me protons (**1a** and **1a·4G**, 2.77 ppm; **2a** and **2a·4G**, 3.67 ppm) in the ^{1}H NMR spectra. The complexation was also shown on the basis of the following NMR experiments. To a $CDCl_3$ solution (0.8 mL) of **1a** (20 μmol) was added **4G** (20 μmol), and ^{1}H NMR spectra were measured. The NH protons on both **1a** and **4G** were shifted downfield by 1.83 (**1a**-NH), 1.25 (**4G**-NH_2), and 0.82 (**4G**-NH) ppm, reflecting the formation of **1a·4G**, and new broad peaks, which might be assigned to NH protons of **2a·4G**, appeared. The association constant (K_s) of 280 ± 20 M^{-1} was determined by the Foster-Fyfe analysis.[8]

As expected, the presence of the cytidine derivative interfered with this selective coloration of **1a** for the guanosine derivative because of the competitive formation of the Watson-Crick G-C base pairs. Thus, addition of **4C** (3.0 equiv to **4G**) to the colored solution caused dramatic fading of the color, but other nucleoside derivatives (**4A**, **4T**, and **4U**) had little influence on it (Figure 1b). While **1b**, which was expected to bind guanine via two hydrogen bonds, also revealed substantially selective coloration for **4G**, the corresponding spiro benzopyran **3** showed no changes in its absorption spectrum in the presence of any nucleoside derivatives.

In summary, we have developed multifunctional artificial receptors for guanosine derivatives, namely, "recognition/structural change/signaling" receptors. In future investigations, the design of receptors which bind native nucleosides and nucleotides is expected to show great practical value.

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Supplementary Material Available: Preparation and spectral data for **1a**, **1b**, **8-12**, and **13** (4 pages). Ordering information is given on any current masthead page.

(8) Foster, R.; Fyfe, C. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1969**, *4*, 1-89. K_s measurements were made by monitoring the chemical shifts of the **4G**-NH proton as a function of **1a** concentration. In such conditions ([**1a**] ≫ [**4G**]), the presence of the opened merocyanines (**2a** and **2a·4G**) was negligible; see text.

Stereospecific Antibody-Catalyzed Reduction of an α-Keto Amide

Grace R. Nakayama and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720

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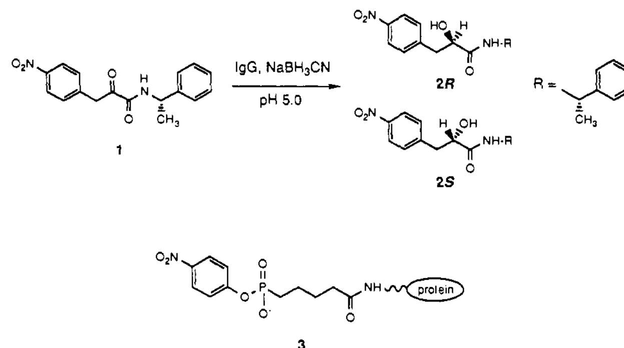
The development of catalytic enantioselective reactions for the synthesis of pure chiral compounds has become an important focus of synthetic organic chemistry.[1] Enzymes are being used in an increasing number of applications by virtue of their remarkable specificities.[2] However, only a limited number of enzymes are available, and in many cases cofactor recycling complicates the use of a biocatalyst. A number of powerful synthetic chiral catalysts have also been developed including epoxidation,[3] hydrogenation,[4] and hydride transfer[5] catalysts. However, the rational design of such catalysts is still at a very early state. An alternate approach to the generation of chiral catalysts exploits nature's ability to generate high-affinity, highly selective receptors by means of the highly evolved machinery of the immune system.[6] We now report the use of antibodies to carry out the catalytic stereospecific reduction of an α-keto amide using the reductant $NaBH_3CN$, a first step toward the generation of a family of catalytic antibodies for chiral alcohol and amine synthesis.

We anticipated that antibodies specific for phosphonate **3** might catalyze the stereospecific, $NaBH_3CN$-dependent reduction of α-keto amide **1** to α-hydroxy amide **2** on the basis of the following considerations: (1) The negatively-charged tetrahedral phosphonate moiety, which can be readily incorporated into haptens, should induce a combining site capable of polarizing a carbonyl group for attack by a hydride reagent. (2) The antibody combining site should provide a chiral environment that discriminates the transition states arising from attack of hydride on the two faces of the carbonyl group.[7] The ability to generate antibodies with any desired specificity (or lack thereof) should ensure the production of antibodies with high enantioselectivities (regioselectivities or substrate specificities). (3) Conjugation of hapten to carrier protein at or near the phosphonate group should ensure accessibility of a relatively small reductant to the carbonyl group (second-generation haptens might incorporate a "reductant site"). Although many enzymes[2] and enzyme mimics[8] for the stereospecific reduction of α-keto acids and their derivatives utilize nicotinamide cofactors, we chose a less expensive, more powerful metal hydride reductant that is capable of reducing a large array of carbonyl groups and carbonyl derivatives.[9]



Monoclonal antibodies specific for phosphonate **3**[10] were purified to homogeneity by affinity chromatography on protein A coupled sepharose[11] as determined by SDS-polyacrylamide gel electrophoresis. Eight antibodies were then assayed for their ability to reduce α-keto amide **1** by high-performance liquid chromatography (HPLC). The (S)-(−)-α-methylbenzylamine group was incorporated into the α-keto acid substrate to facilitate analysis of reaction stereospecificity (it has been previously shown that antibodies specific for phosphonate **3** are relatively insensitive to substitutions in the aliphatic linker[10b]). α-Keto amide **1** was prepared by a modification[12] of the method of Westerberg and co-workers for the preparation of (4-nitrophenyl)pyruvic acid.[13]

* Author to whom correspondence should be addressed.

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(12) Pyruvamide **1** was synthesized from α-acetamido-4-nitrocinnamic acid[13] by treatment with isobutylchloroformate/4-methylmorpholine to form the mixed anhydride, followed by reaction with (S)-(−)-α-methylbenzylamine. Subsequent hydrolysis and chromatography on silica gel (hexanes/i-PrOH) afforded α-keto amide **1**.

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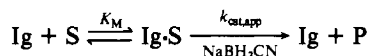
Two of eight antibodies were found to catalyze the reduction of α-keto amide **1** to the α-hydroxy amide **2**. One antibody, A5, was examined in greater detail.

The antibody-catalyzed, $NaBH_3CN$-dependent reduction displayed a pH optimum at acidic pH; consequently, all kinetic parameters were measured in the presence of 13 μM antibody at 22 °C in 50 mM NaCl, 50 mM MES buffer, pH 5.0.[14] The antibody-catalyzed reaction could be described by the following kinetic scheme:



where S is α-keto amide substrate **1**, Ig is antibody A5, K_M is the Michealis constant for **1**, and $k_{cat,app}$ is the k_{cat} (catalytic constant) observed at a particular fixed concentration of $NaBH_3CN$. A Lineweaver–Burk analysis of the steady-state kinetic data at 1 mM $NaBH_3CN$ afforded a k_{cat} of 0.104 min^{-1} and a K_M(**1**) of 1.24 mM. The pseudo-first-order rate constant for the uncatalyzed reaction (k_{uncat}) at 1 mM $NaBH_3CN$ in the same buffer was found to be 3.6×10^{-4} min^{-1}. The antibody-catalyzed reaction was inhibited by 4-nitrophenyl methyl phosphate: the K_d was determined from fluorescence quenching experiments to be 0.61 μM. Greater than 25 turnovers were measured with no apparent change in V_{max}, suggesting that $NaBH_3CN$ does not inactivate the antibody at a significant rate.

The diastereomeric excess of the reaction was determined by extraction of product into methylene chloride followed by acetylation with acetic anhydride/pyridine/DMAP and subsequent analysis using capillary gas chromatography.[16] Product stereochemistry was assigned by comparison to authentic products.[17] Controls demonstrated that the diastereomeric composition of products **2R** and **2S** was stable to the workup and assay conditions. The uncatalyzed reaction afforded α-hydroxy amide **2R** with a diastereomeric excess of 56%. In contrast, the antibody-catalyzed reaction afforded the product **2S** with a diastereomeric excess greater than 99% (*opposite* the stereospecificity of the uncatalyzed reaction), indicating that the antibody combining site discriminates the enantiomeric transition states for carbonyl reduction with high selectivity. Further screening is likely to provide antibodies with a broad array of selectivities including specificity for the product **2R**.

Future experiments will explore antibody-catalyzed, metal hydride dependent carbonyl and imine reductions; regiospecificity; substructure selectivity; and improvements in hapten design (including the use of sulfoxides, phosphinates, and phosphonate diesters). In addition, the use of other powerful synthetic reagents in conjunction with antibody catalysis is being explored.

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(14) Stock solutions of **1** (5 mM) and 10 μL of $NaBH_3CN$ (50 mM) in MeOH were added to 0.5 mL total volume of reaction buffer containing 2 mg/mL antibody and 10% (v/v) MeOH. Initial reaction rates were determined by measuring the amounts of **1** and **2** (both diastereomers) relative to a *p*-cresol internal standard using analytical reverse-phase HPLC (Rainin Dynamax Microsorb C_{18}, 40–80% of 0.06% TFA/CH_3CN in 0.1% aqueous TFA).

(15) The dissociation constant was determined from a Scatchard analysis of fluorescence titrations (λ_{ex} = 280 nm, λ_{em} = 346 nm) using 0.14 mg/mL A5 in assay buffer.

(16) Stock solutions of 20 μL of **1** (5 mM) and 10 μL of $NaBH_3CN$ (50 mM) in MeOH were added to 0.5 mL total volume of 10 mg/mL antibody containing 10% (v/v) MeOH and shaken for 4 h. After extraction of the reaction mixture with CH_2Cl_2 and removal of solvent, the residue was dissolved in CH_3CN and acetylated with acetic anhydride, pyridine, and DMAP. The aceylated products were purified by reverse-phase HPLC (Rainin Dynamax Microsorb C_{18}, 30–80% of 0.06% TFA/CH_3CN in 0.1% aqueous TFA) prior to analysis by capillary GC (HP-1, cross-linked methyl silicone gum, 25 m × 0.2 mm × 0.33 μm film thickness, FID detector), using (*S,S*)-*N*-(α-methylbenzyl)-*O*-acetyl-3-phenyllactamide as an internal standard. The diastereomeric excess of the antibody-catalyzed reaction was corrected for background reaction.

(17) (*S,S*)-*N*-(α-Methylbenzyl)-*O*-acetyl-3-(4-nitrophenyl)lactamide (**2S**) was prepared from (*S*)-(−)-3-(4-nitrophenyl)lactic acid[18] by condensation of the NHS ester with (*S*)-(−)-α-methylbenzylamine followed by acetylation and chromatography on silica gel (CH_2Cl_2/EtOAc). Sodium borohydride reduction of α-keto amide **1** afforded product **2R** (as a mixture of **2R** and **2S**).

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Sugar Conformations in Intramolecular DNA Triplexes Determined by Coupling Constants Obtained by Automated Simulation of P.COSY Cross Peaks

Român F. Macaya, Peter Schultze, and Juli Feigon*

Department of Chemistry and Biochemistry and Molecular Biology Institute University of California, Los Angeles, California 90024

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Evidence for RNA triple helices formed from two pyrimidine strands and one purine strand was first reported in 1957.[1] Subsequently evidence for formation of other RNA and DNA triple helices from polynucleotides was reported by several laboratories.[2] The generally accepted base pairing in these structures was a Watson–Crick duplex formed from one purine and one pyrimidine strand, with the second pyrimidine strand Hoogsteen base paired to the purine strand. Based on fiber diffraction studies of poly(U)·poly(A)·poly(U)[3] and poly(dT)·poly(dA)·poly(dT),[4] Arnott and co-workers concluded that the DNA triplex formed a structure similar to the RNA triplex; that is, an A′ helix with 12 base triplets per turn, an axial rise per residue of 3.26 Å, base tilts of 7–9°, and C3′-endo sugar puckers in all three strands.[4] This model for the structure of DNA triplexes has been widely accepted in the literature.[5] Although the rise per residue and the helical twist can be accurately determined from fiber diffraction data, it should be noted that the sugar conformation cannot be obtained from fiber diffraction due to the low resolution of the data, and the C3′-endo sugar pucker in the triplex model was based on an assumption.[4] In addition, the rise per residue and base tilts are closer to those of B DNA than to those of A′ DNA.[6] In our recent two-dimensional NMR studies on DNA triplexes formed from $d(TC)_4$ and $d(GA)_4$, we confirmed the proposed base-pairing schemes but presented evidence based on NOE intensities that the purine strand did not have N-type (near C3′-endo) sugar puckers.[7] A more reliable estimation of the sugar conformations can only be obtained from analysis of the fine structure of COSY[8] cross peaks.[9] Here we present an analysis of the cross-peak patterns and coupling constants from the phase-sensitive COSY spectrum of a 31-base intramolecular DNA triplex (HD31). Accurate coupling constants were obtained using our new program CHEOPS (*c*oupling constants from *h*igh-resolution

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