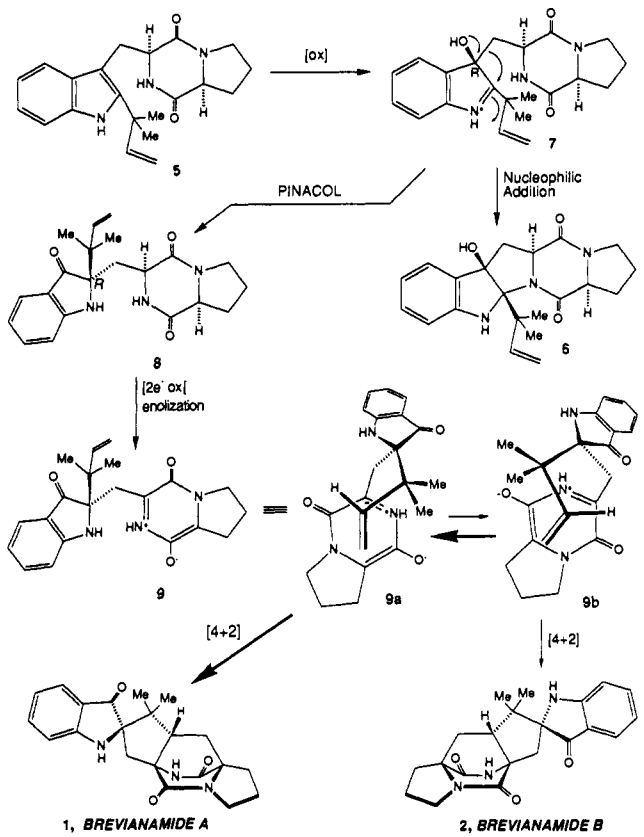
Scheme I



suggest an alternate biosynthetic pathway that is detailed in Scheme I. We presume that, following the conversion of **3** into **5**, an *R*-selective hydroxylation reaction occurs at the 3-position of **5** furnishing **7**. Nucleophilic addition to the C=N bond of **7** leads to **6**. On the other hand, catalyzed pinacol-type rearrangement of **7**[14] sets the *R*-absolute stereochemistry at C-2, to give **8**. This rearrangement justifies the *R*-stereochemistry of the indoxyl, since the 3-hydroxyindolenine **7** is the sterically favored product of oxidation, as shown in the autoxidation of **5**.[12] This is a much more difficult stereochemical issue to rectify via **4** since, experimentally, oxidation of **4** with a peracid proceeds from the least hindered face giving solely **2**.[8] Oxidation of **8** followed by enolization forms the aza diene **9**.[15] An *intramolecular Diels–Alder cyclization* from a major rotamer (**9a**) directly leads to **1**, and a minor rotamer (**9b**) cyclizes to **2**. Molecular mechanics calculations on the rotamers **9a** and **9b** hint that rotamer **9a** is ca. 1 kcal/mol more stable than rotamer **9b**. This is due to a favorable H-bond between the indoxyl ketone and the proximal amide NH of the piperazinedione in **9a**. This proposal accommodates the existence of the two enantiomorphic bicyclo[2.2.2] ring systems, and it also accounts for the preponderance of **1** over **2**.

In conclusion, the intermediacy of **4** in the biosynthesis of **1**/**2** seems unlikely at present. On the other hand, our feeding experiments show that while **5** is a biosynthetic intermediate of both **1** and **2**, **6** is a shunt metabolite which does not lead to these compounds. Studies on the synthesis and possible intermediacy of **8** and **9** are currently in progress in our laboratories.

Acknowledgment. This work was supported by the National Institutes of Health (Grant CA 43969) and the Colorado State University Agricultural Experiment Station as part of USDA SAES Western Project W-122. R.M.W. also acknowledges additional support from Hoffman-La Roche, Inc.; Merck, Sharp & Dohme Research Laboratories; and Symphony Pharmaceutical Co., Inc. J.F.S.-C. thanks the DGICYT of Spain for a fellowship. Technical assistance from Renee Gallegos and James K. Stille is also gratefully acknowledged.

Supplementary Material Available: Details of the biosynthetic feeding and incorporation experiments (1 page). Ordering information is given on any current masthead page.

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Structural Characterization of Organocopper Reagents by EXAFS Spectroscopy

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Organocopper compounds are among the most versatile organometallic reagents for forming new carbon–carbon bonds.[2,3] Although the synthetic utility of these reagents is well established, their reaction mechanisms and their structures remain controversial.[4-7] NMR investigations of their solution structures have revealed the presence of complex equilibria.[5,8] Recently, several

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Table I. First Shell Curve Fitting Results

		I[d]		II[d]	
sample	CN[a]	$R_{Cu-ligand}$[b]	σ^2[c]	$R_{Cu-ligand}$[b]	σ^2[c]
1	3	1.99	2.7	1.97	4.4
2	2	1.91	1.6	1.91	1.7
3	2	1.94	2.8	1.96	2.2

[a] Integer coordination number giving the best fit. [b] Cu–nearest neighbor distance in angstroms. It is not possible to distinguish between Cu–C, Cu–N, and Cu–O scattering in EXAFS. [c] Debye–Waller factor, in units of $Å^2 \times 10^3$. [d] Duplicate data were measured for each complex. I = NSLS and II = SSRL.

important crystal structure determinations of organocopper compounds have been reported.[7] These show that both "lower order" (2-coordinate) and "higher order" (3-coordinate) organocuprate anions can exist in the solid state,[9] and they provide structural details for a variety of lithium-derived organocopper aggregates. Despite extensive study, the structures of cyano-substituted copper reagents, prepared by the reaction of RLi with CuCN, remain particularly unclear, with significant controversy regarding the existence of higher order cuprates.[4-6] No crystal structure of a cyano-substituted organocuprate has been reported,[10] even when CuCN is used as the starting material,[9c] thus calling into question the claim[4] that higher order cuprates represent a thermodynamic sink when cyanide is present.

Extended X-ray absorption fine structure (EXAFS) spectroscopy provides a unique probe of the local structural environment of metal ions in noncrystalline systems.[11] We report herein the results of an EXAFS study of the cyano-substituted copper reagents CuCN+2LiCl,[12] **1**, BuLi+CuCN, **2**,[13] and 2BuLi+CuCN, **3**.[3-5] THF solutions (ca. 0.2 M) of **1–3** were prepared according to literature procedures (supplementary material), loaded into Mylar-windowed Al sample cells, and frozen in liquid N_2. Spectra were recorded in the transmission mode at NSLS beam line X9A and SSRL beam line 7-3 at ca. −195 °C. Data reduction and analysis followed standard procedures.[11,14] Quantitative first shell curve fitting analysis was based on ab initio amplitude and phase parameters,[15] calibrated using model compounds of known structure.

The Fourier transforms of the EXAFS data for **1–3** are shown in Figure 1. These represent pseudo radial distribution functions around the Cu, with peaks shifted by the EXAFS phase shift ($\alpha \approx 0.5$ Å) relative to the true atomic position. In each case, the first shell data are well modeled by a single shell of low-Z scatterers at <2.0 Å (see Table I) with an average coordination number of 3 for **1** and 2 for **2** and **3** (estimated uncertainty ±0.5). Although it is difficult to determine precise coordination numbers from

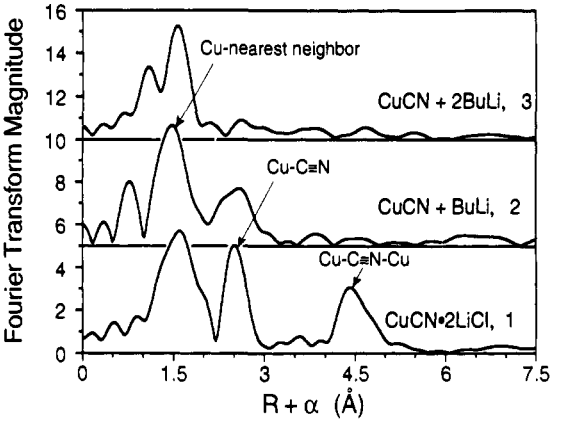

Figure 1. Fourier transform of the EXAFS spectra for compounds **1–3** calculated using k^3 weighted EXAFS from k = 3.5–12.5 $Å^{-1}$. The spectra for **2** and **3** are offset vertically by 5 and 10, respectively, for clarity. Peaks at $R + \alpha \approx 1.5$, 2.5, and 4.5 Å are due to Cu–nearest neighbor, Cu···N (from cyanide), and Cu···Cu scattering, respectively (see text).

EXAFS data, the observed Cu–(C,N,O) distances[16] are consistent with the EXAFS derived coordination numbers. Crystallographically characterized CuR_3^{2-} anions have Cu–C distances of 2.02 Å;[9d,f] thus the short Cu–ligand distances in **2** and **3** support a 2-coordinate assignment. The longer Cu–ligand distance for **1** is typical of 3-coordinate Cu cyanides.[10]

Detection of non nearest neighbor interactions using EXAFS is often problematic. However, for linear geometries (e.g., Cu–C–N) multiple scattering causes a large increase in outer shell EXAFS amplitude.[17] Thus, the Fourier transforms for **1** and solid CuCN (not shown) both have a large Cu···N peak at $R + \alpha \approx 2.5$ Å. A similar peak, with approximately one-half the amplitude, is seen for the lower order cyanocuprate **2**, confirming the recent NMR finding[5b] that cyanide remains coordinated to Cu in **2**. In contrast, the higher order cyanocuprate **3** does not show any outer shell scattering above the noise level (ca. 10%). This description is confirmed by quantitative curve fitting analysis; no improvement was seen in the fit for **3** on including a Cu···N shell. This leads us to the conclusion that the higher order cuprate **3** in THF is better represented by the formula $Bu_2CuLi{\cdot}LiCN$, as proposed by Bertz,[5] than by $Bu_2Cu(CN)Li_2$, since the latter implies coordination of the cyano ligand to copper.[2,3]

A second outer shell peak at $R + \alpha \approx 5$ Å is observed for **1**. This peak, which is also present in CuCN, is due to Cu···Cu EXAFS. Normally Cu···Cu EXAFS would not be detected for such long distance interactions; however, multiple scattering within the linear Cu–C–N–Cu unit again causes significant enhancement of the outer shell amplitude. The presence of this peak for **1** shows that this solubilized CuCN is oligomeric in solution. No comparable Cu···Cu peaks are observed for **2** or **3**, although ca. 2.7 Å Cu···Cu interactions are well documented in organocuprate crystal structures[7,9] (e.g., in $R_2Cu_2Li_2$ dimers). This does not necessarily imply that the cuprates in **2** and **3** are predominantly monomeric, but may simply reflect the decreased detectability of weak Cu···Cu EXAFS in the absence of multiple scattering. Experiments to clarify the detectability of organocuprate Cu···Cu EXAFS are in progress.

The variations in Cu–ligand distance for **1–3**, although small, are larger than the expected error in the EXAFS. The Cu–ligand distances together with the outer shell EXAFS suggest the following structural model. Complex **1** contains $(CuC{\equiv}N)_n$ ($n \geq 2$) oligomers, possibly terminated with lithium and/or chloride ligands. The average coordination number is approximately 3.

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This, plus the presence of the longer Cu-N distances in addition to the shorter Cu—C distances,[18] gives **1** the longest average Cu-ligand distance. On addition of 1 equiv of BuLi the coordination number decreases to 2. This, together with replacement of a relatively long Cu—N≡C distance by a shorter Cu—C(butyl) distance, gives a 0.07 Å decrease in the average bond length. Addition of a second equivalent of BuLi leads to loss of the remaining cyanide, with a small increase in distance as expected on the basis of the difference in size for sp vs sp^3 hybridized C. The average Cu—C distance in **3** is marginally longer than the 1.92-1.94 Å distance found in isolated CuR_2^- units.[9] This may be due to weak secondary interactions between the Cu and the solvent and/or the Li cations, or it may simply reflect the uncertainty in the measurements.

The present data unambiguously show that most of the Cu atoms (>90%) in solution **3** do *not* contain coordinated cyanide, consistent with recent NMR studies.[5] The absence of EXAFS-detectable Cu···N scattering does not, of course, exclude the possibility that a species such as $Bu_2Cu(CN)Li_2$ may be present in **3** in catalytic amounts and be responsible for the higher reactivity of $R_2CuLi{\cdot}LiCN$ compared to $R_2CuLi{\cdot}LiX$ (X = halide).

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Supplementary Material Available: Details of sample preparation (1 page). Ordering information is given on any current masthead page.

(18) In crystallographically characterized Cu cyanides, typical bond lengths are Cu-C = 1.82 Å (1.90 Å) and Cu-N = 1.91 Å (1.97 Å) for 2-coordinate (3-coordinate) complexes.

A Catalytic Antibody for Imide Hydrolysis Featuring a Bifunctional Transition-State Mimic

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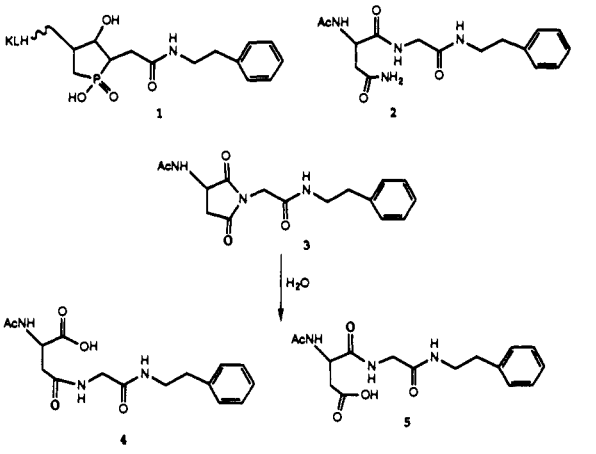
Monoclonal antibodies raised against tetrahedral transition-state analogs have consistently been shown to catalyze acyl transfer reactions.[1] Antibodies generated against **1**, a cyclic phosphinate containing two tetrahedral transition-state mimics, have previously been reported to catalyze the deamination of *N*-acetylasparaginylglycine phenethylamide **2** presumedly through generation of the succinimide intermediate **3**.[2] Since the final product ratio of isoaspartate **4** to aspartate **5** was affected by several of these antibodies, we inferred that they also may catalyze the hydrolysis of **3** (Scheme I). Herein, we report an antibody generated against **1** that is capable of catalyzing the hydrolysis of succinimide **3** by accelerating cleavage at both amide carbonyls. Insight into the functional relationship between the two possible tetrahedral recognition sites on the antibody and the consequences for the regiochemistry of the hydrolysis has been provided by the evaluation of the kinetic parameters for both the D and L isomers of **3** as substrates.

The hapten synthesis and generation of antibodies have previously been reported.[2] Enantiomerically pure succinimides **3** were synthesized from the corresponding asparagines. An initial screen to determine the ratio of the isoaspartate **4** to aspartate **5** products

Scheme I



Scheme II

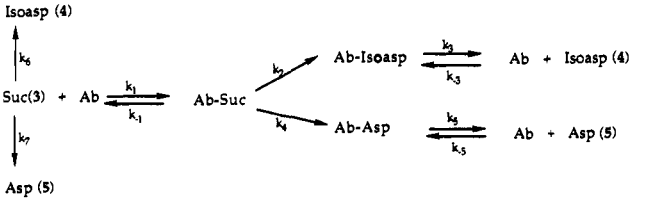


Table I. Kinetic Constants[a] for D- and L-Succinimide **3** Hydrolysis by Antibody RG2-23C7

	D-succinimide	L-succinimide
k_{-1}/k_1	0.24 μM	0.83 μM
k_2(isoasp)	0.52 min^{-1}	3.5 min^{-1}
k_4(asp)	0.56 min^{-1}	0.12 min^{-1}
k_3/k_{-3}(isoasp)	0.24 μM	0.25 μM
k_5/k_{-5}(asp)	0.14 μM	0.10 μM
k_2/k_6	70.3	486
k_4/k_7	287	61.5

[a] ±10%.

was performed by HPLC.[3] An antibody designated RG2-23C7 among a grouping of 30 antibodies produced the largest deviation from background in the ratio of **4** to **5**. Ratios of 10.9 for the L-succinimide and 1.5 for the D-succinimide were found as compared to 3.7 for the background reaction. This antibody was then subjected to further kinetic analysis.

The full reaction course was followed by HPLC, and the data were fit to Scheme II using computer simulations of the reaction time course (KINSIM).[4] The product dissociation constants for the D- and L-aspartate **5** and the D- and L-isoaspartate **4** (k_5/k_{-5} and k_3/k_{-3}, respectively) were determined by fluorescence titrations.[5] The succinimide dissociation constant (k_{-1}/k_1) and the catalytic rate constants (k_2 and k_4) were then determined from computer-generated fits to the HPLC data. The kinetic constants for the hydrolysis of both the L- and D-succinimides thus obtained are given in Table I. Since the fluorescence changes observed during the titrations were small, the strong product inhibition was verified by conducting the hydrolysis of **3** in the presence of an initial 1 mM concentration of either **4** or **5** and showing that the product data was fit by the corresponding simulations.

Evaluation of the kinetic constants indicates that RG2-23C7 binds the D isomer about 4 times better than it binds the L isomer. Since the antibodies were produced in response to a racemic mixture, this result implies that the true hapten was the D isomer of phosphinate **1**. It has previously been shown that catalytic

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