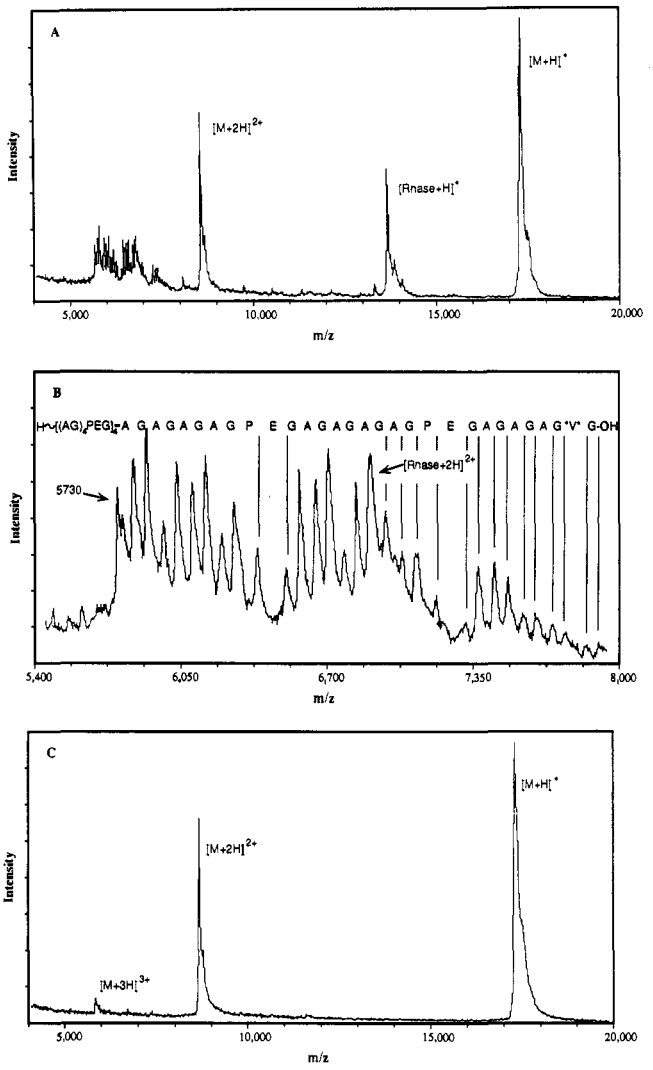

Figure 1. Analysis of artificial proteins by matrix-assisted laser desorption mass spectrometry. (A) Spectrum of target copolypeptide **1** with RNase A as an internal calibrant. The m/z value of the molecular ion $[M + H]^+$ is 17264 ± 2. (B) Spectrum expanded in region of low molecular weight contaminants. The peaks progress starting with N-terminal fragment **3** (calcd m/z = 5733, found 5730) with the sequential addition of amino acid residues through three repeats of the target repetitive sequence. The substituted valine is apparent in the seventh repeat. (C) Mass spectrum of the protein sample after removal of the contaminating fragments by dialysis.

3 is 5733, in good agreement with the observed value. More striking, however, is the fact that each succeeding signal can be rationalized by addition of a single amino acid residue, proceeding in the N- to C-terminal direction along sequence **1**. Thus, one can read portions of the periodic sequence directly from the mass spectrum, including one of the substituted valines. These fragments appear in all preparations of the copolypeptide and probably arise from the action of exo- and endopeptidases, either in vivo and in vitro or both. The fragments are easily removed by dialysis as shown in Figure 1c, which shows only the singly, doubly, and triply ionized molecular ions of the intact protein. Fragmentation is not an artifact of the ionization technique.

H-ASMTGGQQMGRDPMFKYSRDPMG-[AGAGAGAGPEG]$_4$-OH (**3**)

Subsequent to the discovery of the contaminating fragments, the sample was rerun on a 25-cm 15% SDS polyacrylamide gel, and the proteins were visualized by Coomassie Blue staining. The fragments were not detected by this method, even when the sample was overloaded (up to 50 μg of protein per lane). In contrast, matrix-assisted laser desorption mass spectrometry provides rapid, accurate determination of molecular weight, reveals minor protein contaminants, and provides direct amino acid sequence information.

Acknowledgment. This work was supported by grants from the National Science Foundation (DMR 8914359) and the National Institutes of Health (RR00862, GM38724, and BRSG S07 RR07065).

A Substoichiometric Pyridine–Lithium Enolate Complex: Solution and X-ray Data and Implications for Catalysis in the Aldol Reaction

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The coordination chemistry of lithium enolates has been the subject of extensive investigation,[1-4] and the relative reactivity of different aggregates has emerged as an area of central concern.[5] In the context of a broad approach to the design of asymmetric catalytic aldol reactions, we are investigating the effect of substoichiometric amounts of Lewis bases on the aggregation state and reactivity of enolates. Enantioselective catalysis is achievable in principle with chiral ligands that enhance enolate reactivity, effectively induce asymmetry in the aldol condensation, and turn over due to preferential binding to free enolate relative to the metal aldolate product.[6] We report herein our initial studies directed toward this goal, including the unexpected reaggregation of lithium pinacolate (**1**) with substoichiometric amounts of pyridine and the first definitive observation of a coordinatively unsaturated lithium enolate complex.[7]

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(2) For reviews on lithium enolates: (a) Jackman, L. M.; Lange, B. C. *Tetrahedron* **1977**, *33*, 2737. (b) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988** *27*, 1624. (c) Jackman, L. M. In *Comprehensive Carbanion Chemistry*, in press.

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(5) (a) Jackman, L. M.; Dunne, T. S. *J. Am. Chem. Soc.* **1985**, *107*, 2805. (b) Williard, P. G.; Hintze, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 8602. (c) Williard, P. G.; MacEwan, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 7671. (d) Hall, P. L.; Gilchrist, J. H.; Collum, D. B. *J. Am. Chem. Soc.* **1991**, *113*, 9571 and references cited therein. (e) Hall, P. L.; Gilchrist, J. H.; Harrison, A. T.; Collum, D. B. *J. Am. Chem. Soc.* **1991**, *113*, 9575. (f) Jackman, L. M.; Rakiewicz, E. F. *J. Am. Chem. Soc.* **1991**, *113*, 4101 and references cited therein.

(6) Arnett has previously demonstrated that Lewis bases such as THF and TMEDA bind to lithium enolates yet do not interact measurably with the corresponding aldolates. Arnett, E. M.; Fisher, F. J.; Nichols, M. A.; Ribeiro, A. A. *J. Am. Chem. Soc.* **1989**, *111*, 748.

(7) Although the ligand-free hexamer **1** is formally coordinatively unsaturated, Williard noted that the unusually short contacts between the lithium centers and the enolate double bonds may reflect a weak dative interaction. The resulting stabilization was proposed to account for the hexameric structure, since such chelation is geometrically less accessible in a tetramer. Williard, P. G.; Carpenter, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 3345.

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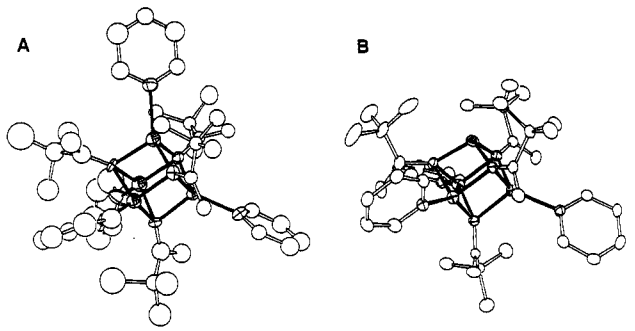

Figure 1. ORTEP drawings of A and B. The Li–O tetrameric core and the N–Li bonds are highlighted. Thermal ellipsoids are scaled to represent 35% probability surfaces.

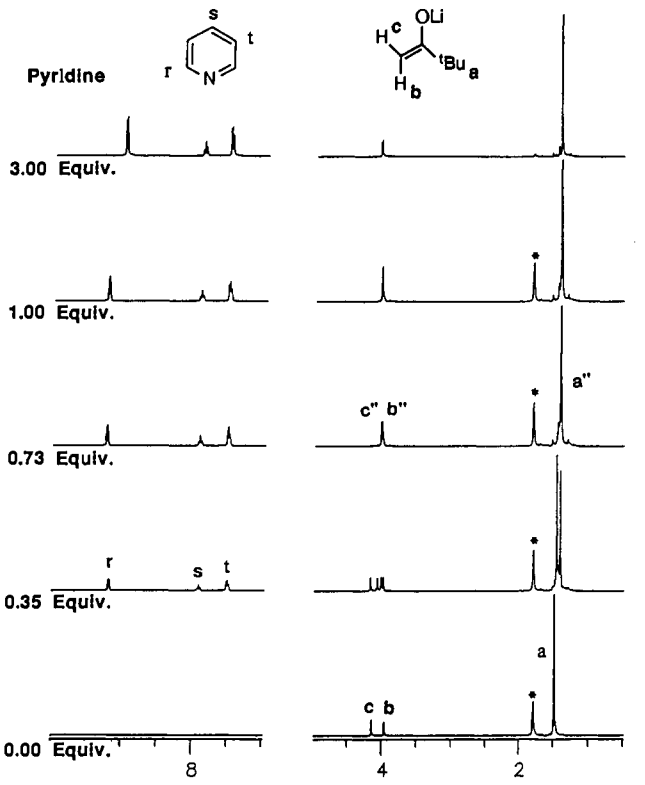

Figure 2. ^{1}H NMR titration plot of a lithium pinacolate (**1**) solution (0.1 M) in cyclohexane-d_{12} with pyridine at 20 °C; a, b, and c correspond to resonances of free enolate; a″, b″, and c″ are the resonances due to the pyridine–enolate complex; and r, t, and s correspond to pyridine; (*) $C_6D_{11}H$.

Treatment of pinacolone with lithium bis(trimethylsilyl)amide in the presence of excess pyridine afforded an isolable lithium pinacolate complex, the solid-state structure of which was determined by X-ray crystallography to be the 4:4 enolate–pyridine tetramer A (Figure 1). This structure is closely analogous to the corresponding THF complex reported by Seebach and Dunitz.[8] Thus **1**, which is a hexamer in both solution and the solid state,[4a,7] undergoes clean reaggregation to Lewis base-complexed tetramers upon addition of pyridine or THF.

Titrations of 0.1 M cyclohexane-d_{12} solutions of either **1** or the lithium aldolate **2**[9] with Lewis bases were monitored by ^{1}H NMR. Whereas aldolate **2** underwent no detectable binding to pyridine nor to a wide range of other potential ligands,[6] titration of **1** with a variety of oxygen- or nitrogen-containing Lewis bases resulted in dramatic changes in ^{1}H NMR signals of the enolate. In the titration with pyridine at 20 °C (Figure 2), the resonances due to **1** (δ 4.13, 3.95, 1.46) were replaced by a distinct set of enolate peaks (δ 4.00, 3.98, 1.38). Complete disappearance of **1** occurred with only 0.74 equiv of added pyridine (Figure 2), whereas a full 1 equiv of added THF was required to achieve complete reaggregation of the enolate at 20 °C.

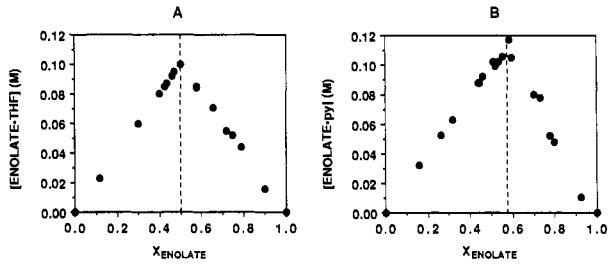

Figure 3. Job plots. (a) Plot of [enolate–THF] versus the mole fraction [**1**]/([**1**] + [THF]) at constant [**1**] + [THF] = 0.2 M (20 °C). The dashed line at $X_{enolate}$ = 0.5 indicates the predicted position of the curve apex for a 4:4 enolate–THF complex. (b) Plot of [enolate–pyridine] versus the mole fraction [**1**]/([**1**] + [pyridine]) at constant [**1**] + [pyridine] = 0.2 M (20 °C). The dashed line at $X_{enolate}$ = 0.57 indicates the predicted position of the curve apex for a 4:3 enolate–pyridine complex.

The difference in the binding behavior of THF and pyridine was investigated further using Job's method of continuous variations.[10,11] The apex of the Job plot for the interaction of THF with **1** occurred at an equimolar ratio of enolate to ligand ($X_{enolate}$ = 0.5), as expected for 1:1 binding (Figure 3A). In contrast, the Job plot of the interaction of pyridine with **1** reached a maximum concentration of complex at an enolate:pyridine ratio of 1.00:0.75 ($X_{enolate} \approx 0.57$), indicating a complex of 4:3 stoichiometry (Figure 3B). The solution aggregation state of this complex was assessed by freezing point depression studies in cyclohexane.[12] A molecular weight of 674 ± 30 g/mol thus determined from solutions containing 0.1 M **1** and 0.74 equiv of pyridine is consistent with an enolate tetramer bearing three pyridine ligands ($MW_{theoretical}$ = 662).

Crystals of the nonstoichiometric enolate–pyridine complex B were grown from a mixture of pinacolone, lithium bis(trimethylsilyl)amide, and pyridine (1:1:0.65) in methylcyclohexane. The solid-state structure was determined by X-ray crystallography (Figure 1) to be that of a tetramer bearing one tricoordinate lithium site, consistent with the solution measurements described above.[13,14] One pyridine ligand is bound to each of the three remaining lithium centers, and these groups are oriented similarly to those of the 4:4 complex. The enolate attached opposite to the open lithium coordination site of the tetramer is also positioned as in the 4:4 complex. The average distance between the bare lithium and the adjacent enolate oxygens is 1.89 Å, whereas the average lithium–oxygen distance is 1.97 Å in the saturated 4:4 complex. It therefore appears that the open lithium site is partially stabilized by stronger interaction with the adjacent enolate oxygens.

The bare lithium site in the 4:3 complex is also shielded by the three *tert*-butyl groups of adjacent enolates, and reorientation of

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(14) Williard and co-workers have recently elucidated the structures of *n*-BuLi tetramers that are partially solvated by TMEDA. Williard, P. G. Personal communication.

one of the enolate groups would be required for a closed transition state aldol reaction involving the tetramer.[15] Molecular mechanics calculations (MM2) performed on the 4:3 complex indeed indicate unhindered rotation of the enolate ligands, so coordination of a fourth group is expected to be sterically accessible without prior deaggregation of the tetramer.[16] Seebach has proposed that aldol reactions of tetrameric Lewis base-coordinated enolates may take place via a ligand dissociative process, and although our data do not provide definitive evidence for such a mechanism, they certainly support the viability of a coordinatively unsaturated reactive tetramer.[17] On the basis of these results, experiments to evaluate the relative reactivities of the ligand-free, 3:4, and 4:4 complexes[18] and of related lithium enolates bearing chiral ligands are currently underway in our laboratories.

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Supplementary Material Available: Experimental procedures, titration procedures, and details of the aggregation measurements and X-ray diffraction studies (35 pages). Ordering information is given on any current masthead page.

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Miyakolide: A Bryostatin-like Macrolide from a Sponge, *Polyfibrospongia* sp.

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We recently discovered that a sponge of the genus *Polyfibrospongia* contained a novel class of bis-oxazoles, one of which, hennoxazole A, displayed significant antiviral activity.[1] This finding prompted us to examine the sponge further to determine whether other biologically active constituents were present. We now report on the isolation and characterization of a new macrolide, miyakolide (**1**),[2] which is structurally similar to the bryostatins, an important class of anti-cancer compounds.[3]

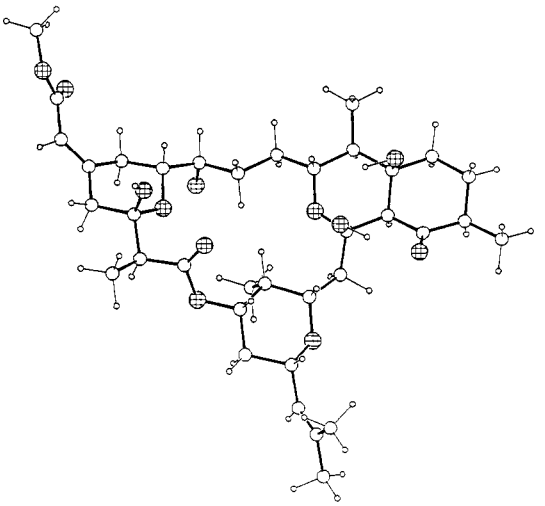

Figure 1. Perspective drawing of the relative configuration of miyakolide (**1**) as determined by X-ray analysis. The oxygen atoms are hatched.

Polyfibrospongia sp. (8.4 kg) was collected at the same site off the island of Miyako and processed in the same manner as previously described.[1] The fractions obtained by initial chromatography, which showed TLC spots different from those of the hennoxazoles, were separated by Sephadex LH-20 (CH_2Cl_2/MeOH 1:2). The resulting fractions were repeatedly separated by HPLC (RP-8, MeOH/H_2O 5:1; Si-60, hexane/EtOAc 3:2) to give 97 mg (0.0012%) of miyakolide (**1**).[4] Recrystallization (CH_2Cl_2/MeOH) furnished colorless crystals: mp 197-199 °C; $[\alpha]^{24}_D$ -24° (*c* 1.05, $CHCl_3$). The molecular formula $C_{36}H_{54}O_{12}$ was established by HRFABMS on a fragment ion at *m/z* 661.3606 (M - OH, $C_{36}H_{53}O_{11}$ requires 661.3624). The presence of four rings in **1** was inferred from the unsaturation requirement of the molecule and from the ^{13}C NMR data,[4] which pointed to a ketone (δ 215.0), two ester carbonyl functions (δ 176.0, 166.5), and two C=C double bonds (δ 154.5, 136.1, 124.9, 116.9).

Analysis of the ^1H, ^{13}C, and 2D spectra (H-H and C-H COSY, COLOC) enabled the structures of rings A-D and their substituents to be deduced. All connections, except that between rings B and D for which there are two possibilities, were also established by 2D NMR spectroscopy. Since this ambiguity could not be resolved by the NMR method, recourse was made to X-ray analysis. A suitable crystal was grown from a solution in Et-

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(2) The compound was named after the island Miyako where the sponge was collected.
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(4) **1**: UV (MeOH) λ_{max} 225 nm (ε 21 000); IR (KBr) 3450, 2930, 1705, 1650, 1190, 1150 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.74 (1 H, s, H34), 5.18 (ddd, *J* = 12, 5, 5 Hz, H13), 5.15 (1 H, d, *J* = 8.3 Hz, H29), 5.02 (1 H, d, *J* = 2 Hz, C18-OH), 4.48 (1 H, s, C9-OH), 4.13 (1 H, ddd, *J* = 10, 9, 2 Hz, H15), 3.88 (1 H, br d, *J* = 10 Hz, H11), 3.84 (1 H, dd, *J* = 12, 1 Hz, H22), 3.74 (1 H, d, *J* = 13 Hz, H21), 3.63 (3 H, s, OCH_3), 3.54 (1 H, dd, *J* = 11, 11 Hz, H1), 3.51 (1 H, d, *J* = 1 Hz, OH), 3.40 (1 H, dd, *J* = 10, 10 Hz, H23), 2.70 (1 H, q, *J* = 7 Hz, H17), 2.56 (1 H, s, H8), 2.48 (1 H, m, H6), 2.47 (1 H, m, H12), 2.42 (1 H, d, *J* = 13 Hz, H19), 2.34 (1 H, dd, *J* = 13, 12 Hz, H21), 2.09 (1 H, d, *J* = 13 Hz, H19), 2.02 (1 H, br d, *J* = 12 Hz, H4), 1.98 (1 H, m, H10), 1.96 (1 H, m, H5), 1.76 (2 H, m, H24, H5), 1.70 (1 H, m, H10), 1.70 (3 H, s, H31), 1.68 (1 H, m, H14), 1.68 (3 H, s, H32), 1.62 (1 H, m, H25), 1.52 (1 H, M, H14), 1.49 (1 H, m, H2), 1.48 (1 H, m, H25), 1.47 (1 H, m, H4), 1.22 (3 H, d, *J* = 7 Hz, H33), 1.21 (1 H, m, H24), 0.96 (3 H, d, *J* = 7 Hz, H27), 0.92 (3 H, d, *J* = 7 Hz, H28), 0.86 (3 H, d, *J* = 7 Hz, H26); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 215.0 (C7), 176.0 (C16), 166.5 (C35), 154.5 (C20), 136.1 (C30), 124.9 (C29), 116.9 (C34), 99.4 (C18), 96.1 (C9), 78.9 (C3), 73.6 (C11), 73.6 (C23), 73.4 (C22), 73.3 (C15), 73.2 (C13), 71.2 (C1), 60.4 (C8), 50.9 (OCH_3), 47.4 (C17), 45.6 (C6), 43.9 (C2), 42.9 (C19), 41.1 (C10), 34.3 (C4), 34.3 (C12), 32.9 (C24), 32.3 (C5), 31.7 (C14), 31.6 (C21), 30.5 (C25), 25.6 (C31), 18.3 (C32), 13.7 (C27), 12.5 (C33), 10.0 (C26), 5.6 (C28); LR FABMS *m/z* 678 (M^+), 677, 661, 307, 289.

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