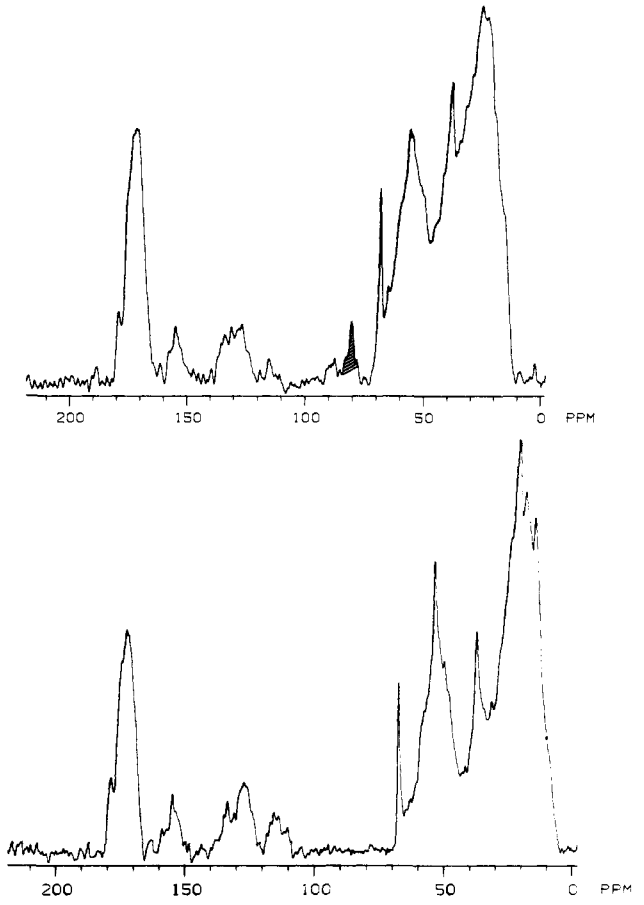

Figure 1. (A, top) Proton-decoupled 50-MHz ^{13}C NMR spectrum (4000 scans) of 110–112 mg/mL aldolase (2.8 mM subunits) in the presence of 3.6 mM [1-^{2}H,1-^{13}C]glycolaldehyde phosphate (**2**) in 5 mM triethanolamine·HCl buffer, pH 7.4–7.7, at 8 °C. Sharp peaks at 67.4 and 53.3 ppm represent dioxane and buffer, respectively. (B, bottom) Spectrum of the enzyme alone (12 000 scans).

nolamine rather than a hydrate by the following considerations. First, the 9.8-ppm change in chemical shift upon binding of **2** appears to be too large for a simple change in the environment of the hydrate due to noncovalent binding. In the interaction of hydroxyacetone phosphate with aldolase, which does appear to be predominantly noncovalent,[14] chemical shifts of carbonyl and hydrate resonances were altered by no more than 0.1 ppm.[6] Second, the dissociation constant of glycolaldehyde phosphate is comparable to that of dihydroxyacetone phosphate, which is known to form predominantly covalent complexes, whereas noncovalent binding of monophosphate esters is typically associated with dissociation constants that are 2–3 orders of magnitude larger.[14,15] Third, the chemical shift of the resonance of bound **2** changes by 0.5 ppm between pH 7.1 and 9.4,[6] which is within the observed range of pH effects on α-carbon chemical shifts of protonated and unprotonated alkylamines.[16]

The enzyme-bound carbinolamine is presumably stabilized by interactions with amino acid residues at the active site, because carbinolamines do not predominate in the nonenzymic reaction of glycolaldehyde phosphate with amines.[17] Since similar interactions may stabilize carbinolamine-like transition states at the active site, the residues may also play a catalytic role in the interconversion between free substrate and enzyme-bound imine. It is interesting that formation and breakdown of an aldolase–dihydroxyacetone phosphate imine appears to be at least partially rate limiting in the overall reaction of fructose 1,6-bisphosphate.[15a,c] This suggests that the aldolase–glycolaldehyde phosphate carbinolamine may represent a transition-state analogue of the overall reaction, provided that glycolaldehyde phosphate and dihydroxyacetone phosphate interact with the enzyme by the same catalytic mechanism, and may account for the tight binding of the partial substrate, glycolaldehyde phosphate.

Acknowledgment. This research was supported in part by the Grace M. Showalter Foundation. The NMR experiments were carried out at the Purdue University Biochemical Magnetic Resonance Laboratory, which is supported by NIH Grant RR-01077. We thank the laboratory staff for patient instruction and assistance. We thank Prof. B. D. Nageswara Rao and Dr. E. L. Ferroni for helpful discussions.

Registry No. 1, 85710-91-4; **2**, 85710-92-5; fructose 1,6-bisphosphate aldolase, 9024-52-6.

(13) Inactivation of the complex by borohydride ion implies that some imine is in relatively rapid equilibrium with the carbinolamine. The noise level of the spectrum in Figure 1A (carbinolamine S/N = 8.1) could mask as much as 34% of imine, if it were represented by an undetectable resonance (S/N <2) with a line width equal to that of the carbinolamine resonance. The possibility that a larger quantity of imine might have been concealed by a broader resonance was considered unlikely because (a) the ratio of peak areas of ^{13}C-enriched and protein carbonyl resonances in Figure 1A (0.039) was comparable to that (0.042) in the spectrum of a borodeuteride-reduced aldolase–[2-^{13}C]dihydroxyacetone phosphate complex;[6] (b) no hidden broad bands were revealed by apodization of the free-induction decay signal of Figure 1A with progressively larger line widths (≤150 Hz).

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(17) Aqueous solutions containing 3.3 mM **1** and excess butylamine or (2,2-dimethoxyethyl)amine (pH > pK_a), exhibited resonances due to enriched carbon at 165–170 ppm ($^{3}J_{CP}$ = 8.4 Hz; imine) and 90.3 ppm ($^{3}J_{CP}$ = 7.3 Hz; hydrate). Resonances attributable to carbinolamine (70–90 ppm) were not seen at any pH value.

Synthetic Studies on the Taxane Diterpenes. Utility of the Intramolecular Diels–Alder Reaction for a Single-Step Stereocontrolled Synthesis of a Taxane Model System

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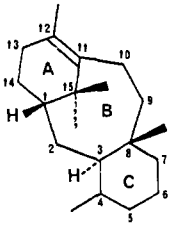
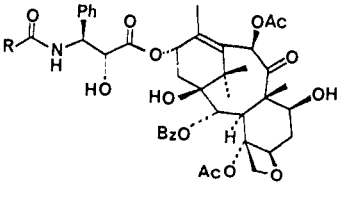
The taxane diterpenes,[1] isolated from various species of *Taxus*, possess the unusual tricyclic carbon framework **1**[2] containing a





1

1a $\Delta^{4,5}$-9-oxo-derivative of **1**

2 R = Ph

3 R = CMeCHMe

sterically congested eight-membered B ring. Some of these such as taxol[3] (**2**) and cephalomannine[4] (**3**) exhibit highly promising

(1) For a review see: Miller, R. W. *J. Nat. Prod.* **1980**, *43*, 425.

(2) Among approximately 30 naturally occurring taxane diterpenes presently known,[1] the tricyclic skeleton **1** is common to all except two. For the exceptions, see: Chiang, H. C.; Woods, M. C.; Nakadaira, Y.; Nakanishi, K. *Chem. Commun.* **1967**, 1201.

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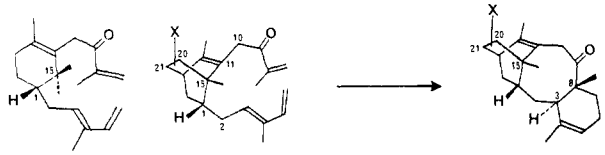
antileukemic and antitumor properties. Because of the unusual structure and biological and biogenetic[5] importance, the taxane diterpenes have attracted the interest of synthetic chemists for nearly a decade.[6-8] However, methodologies capable of constructing even the basic framework **1** (or its equivalent) by a totally synthetic pathway have not been developed. We report a method that allows a single-step stereocontrolled synthesis of a taxane model system (**6a**) from a suitable precursor (**5a**) utilizing an intramolecular Diels–Alder reaction.[9]



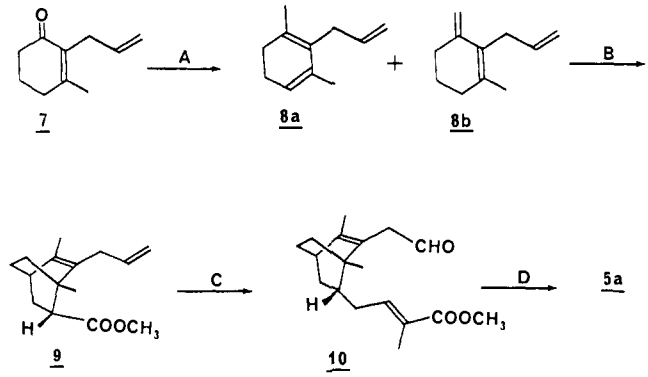
4 **5** **6**

5a X = H **6a** X = H

6b C8 epimer of **6a**

The central problem in taxane diterpene synthesis is, as expected[10] and amply shown,[6-7] the stereocontrolled synthesis of the B ring (cf. **1**). Particularly, the extreme difficulty of direct closure of this ring has been well demonstrated.[6] We hoped to develop a useful direct cyclization method based on the intramolecular Diels–Alder reaction, e.g., in **4** → **1a**. However, there are two potential problems in this seemingly straightforward approach: the unfavorable conformation of **4** for **4** → **1a**,[11] and the known[9] difficulty of eight-membered ring syntheses by intramolecular Diels–Alder reactions.[12] Therefore, we considered it crucial to design the substrate structure to attain maximum entropic assistance for the cyclization. We chose as the key substrate **5**, in which the five carbon atoms C2, C1, C15, C11, and C10 are rigidly positioned by the boat A-ring conformation[13] so as to facilitate the desired closure **5** → **6**. Additionally, potential complications involving intermolecular processes might be minimized by the steric congestion in **5**. With provision of an appropriate functionality X at C20 or C21,[14] the bicyclo[2.2.2]octene system of **5** and **6** can be considered as synthetically equivalent to the taxane A ring. From molecular models, we expected the regio- and stereoselective formation of **6** from **5**.

Scheme I[a]



7 **8a** **8b**

9 **10** **5a**

[a] Key: (A) MeMgBr/THF (0 °C), $MgSO_4$/pentane (25 °C; overall 95% as a 1:1 mixture of 8a and 8b); (B) (1:1 mixture of 8a and 8b)/ $CH_2CHCOOMe/AlCl_3/PhH$ (25 °C)[15c] [60%]; (C) $LiAlH_4$/THF (0 °C), MsCl/THF/Et_3N (0 °C), NaCN/NaI/DMSO (80 °C), Dibal/CH_2Cl_2 (−70 → 0 °C) [overall 95%], $Ph_3PCMeCOOMe/PhH$ (25 → 65 °C) [83%], OsO_4/THF (−20 °C) [48% as a diastereomeric 1:1 mixture], $NaIO_4$/aqueous MeOH (0 °C) [86%]; (D) CH_2-CMeMgBr/THF (−75 °C), *t*-$BuMe_2SiCl$/DMAP/DMFA (25 °C) [overall 65% as a diastereomeric 1:1 mixture], dibal/CH_2Cl_2/hexane (−75 °C), PDC/DMFA (0 °C), CH_2PPh_3/THF (0 °C), *n*-Bu_4NF/THF (25 °C) [overall 78% as a diastereomeric 1:1 mixture], TFAA/DMSO/CH_2Cl_2 (−70 °C) followed by $(i\text{-}Pr)_2EtN$ (−70 → 0 °C) [95% based on consumed material].

These ideas have been tested on the model compound **5a**,[15a] which was prepared from **7**[15b] as shown in Scheme I.

For the catalyzed[16] reactions of **5a**, Me_2AlCl was found to be most effective. In the presence of Me_2AlCl (3 equiv) in benzene, **5a** was smoothly converted at 25 °C into a mixture (95:5 by 300-MHz NMR) of two products in 90% yield. The structure of the predominant product, isolated as crystals [mp 111.0–111.5 °C from aqueous ethanol; IR (KBr) 1691 cm^{-1}], was shown by X-ray crystal structure determination[17a] to be **6b**, the C8 epimer of the expected **6a**.

Thus, we investigated the uncatalyzed reactions of **5a**. Under the initial conditions studied (160 °C, degassed sealed tube, toluene as solvent), we were unable to isolate the two major products formed (about 4:1 detected by thin-layer chromatography) due to contamination of minor byproducts. However, the addition of 5 equiv of trimethyl borate almost completely suppressed the byproduct formation without noticeably affecting the rates of the two major product formations (4:1). The predominant product, isolated by chromatography in 70% yield, was recrystallized from aqueous ethanol [mp 146.5–147.0 °C; IR (KBr) 1680 cm^{-1}]. X-ray crystal structure determination[17a] has established this to be the desired **6a**. In crystals of **6a** and **6b**, the B rings have the preferred boat–chair conformation[13a,17b] with the shortest transannular distances between C3 and C11.

While we are gratified to demonstrate the utility of intramolecular Diels–Alder reactions for direct construction of the bicyclo[6.4.0]dodecane systems[12] that are incorporated in **6a** and **6b**, we are also surprised by the remarkable difference in the stereochemical outcome between the catalyzed and uncatalyzed conditions. Our current efforts are directed toward total synthesis of the taxane diterpenes as well as to illuminate the origin of the contrasting results of the Diels–Alder reactions.

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(11) Axial orientation of the C1 diene substituent is required for **4** → **1a**. The presence of the C15 geminal methyl groups in **4** is expected to disfavor the cyclization: Boeckman, R., Jr.; Ko, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1033 and references cited therein.

(12) To our knowledge, successful synthesis of a bicyclo[6.4.0]dodecane ring system by the application of an intramolecular Diels–Alder reaction has not been reported.

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Acknowledgment. We are grateful for support to K.S. from Carnegie-Mellon University through a grant from Ford Motor Co. and to B.M.C. from the National Institutes of Health (HL20350). We thank Dr. R. F. Stewart for fostering this collaborative effort. Crystallographic calculations were carried out by using the computer programs written or modified by Dr. Shiono. The 300- and 600-MHz NMR spectra (included in the supplementary material) were obtained by using facilities supported by the National Institutes of Health (GM27390, RR00272). We are indebted to Drs. A. A. Bothner-By, M. Llinas, R. Stephens, and J. Lecomte for their help in obtaining the spectra.

Registry No. (±)-**5a**, 85710-89-0; (±)-**6a**, 85710-90-3; (±)-**6b**, 85760-86-7.

Supplementary Material Available: NMR spectra for synthetic substances (19 pages). Ordering information is given on current masthead page.

Reactions of *tert*-Butyl Isocyanide with a Binuclear Niobium(III) Compound

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In the emerging chemistry of niobium and tantalum in their lower oxidation states, but without η^5-C_5R_5 groups, there is already evidence for remarkable reactivity toward nitriles,[1-3] acetylenes,[3-7] olefins,[8] and hydrogen.[8-10] We have now found that very remarkable compounds can be obtained by using isocyanides, e.g., by reaction of t-C_4H_9NC, with $Nb_2Cl_6(SMe_2)_3$ (Nb═Nb),[11] and we here describe two of these. The product yields are strongly influenced by reaction conditions, including the mole ratio of reactants. With an isocyanide/$Nb_2Cl_6(SMe_2)_3$ ratio greater than 7:1 two compounds, having the following apparent stoichiometries, are obtained: $Nb_3Cl_8(t\text{-BuNC})_5$ (**1**) and $Nb_2Cl_6(t\text{-BuNC})_6$ (**2**). These two compounds, neither of which can be converted into the other, probably arise from a common (as yet unidentified) green precursor. Crystalline red-brown compound **1** is obtained (ca. 20% yield) admixed with an amorphous dark solid by very slow interdiffusion of solutions of the reactants, whereas purple compound **2** is formed (ca. 60% yield) by rapid mixing of the solutions. To obtain good crystals of **2**, it was redissolved in 3:1 mixture of Et_2O and CH_3CN and the solution cooled to 0 °C.

Crystal structure determinations were carried out by standard methods, using data from an automated counter-diffractometer.[12,13]

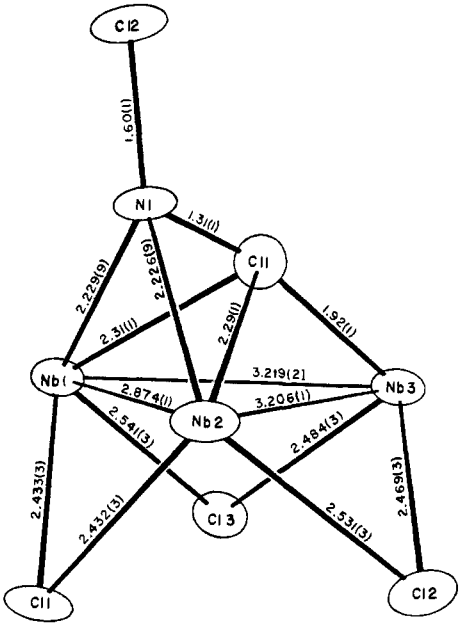
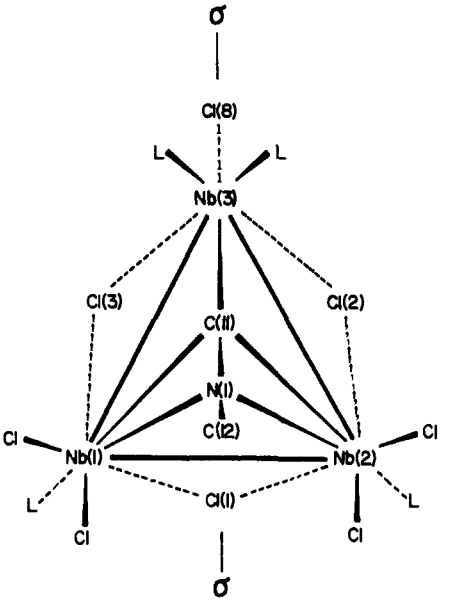


Figure 1. Central portion of the $Nb_3Cl_8(t\text{-BuNC})_5$ molecule, showing important bond lengths.

In neither case is any crystallographic symmetry imposed on the molecules.

The $Nb_3Cl_8(t\text{-BuNC})_5$ molecule, shown schematically in I, has



an approximate symmetry plane passing through Nb(3), Cl(8), C(11), N(1), C(12), and Cl(1) and bisecting the Nb(1)-Nb(2) bond. The more important bond lengths are shown in the ORTEP view of the central portion of the molecule, Figure 1. The niobium atoms define an isosceles triangle, with a bridging Cl atom below each edge and a rare type of multiply bridging isocyanide above the metal atom triangle. The short metal-metal distance (Nb(1)-Nb(2), 2.874 (1) Å) is suggestive of a two-electron bond, while the interpretation of the other two Nb-Nb distances, which average 3.213 [9] Å, is problematical. Nor is a detailed description of the bonding of the multiply bridging t-BuNC molecule to the Nb_3 cluster unambiguous solely on the basis of structural data.

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(12) Compound **1** crystallizes with two $C_6H_5CH_3$ per Nb_3 unit in space group $P2_1/c$ with unit cell dimensions of a = 23.750 (6) Å, b = 9.099 (2) Å, c = 25.031 (3) Å, β = 95.59 (2)°, and Z = 4 (trinuclear units). Refinement of 375 parameters using 3538 independent reflections with $F^2 \geq 3\sigma(F^2)$ produced reliability indices of R_1 = 0.071 and R_2 = 0.085. Tables of atomic positional and thermal parameters are available as supplementary material.
(13) Compound **2** crystallizes in space group Pn with unit cell dimensions of a = 14.102 (2) Å, b = 13.486 (5) Å, c = 11.157 (4) Å, β = 93.26 (2)°, and Z = 2 (dinuclear units). Refinement of 392 parameters using 2438 independent reflections with $F^2 \geq 3\sigma(F^2)$ produced reliability indices of R_1 = 0.054 and R_2 = 0.073. Tables of atomic positional and thermal parameters are available as supplementary material.

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