analysis of Fisher et al.[14] for protein-protein complexes.

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Note Added in Proof. After acceptance of this manuscript for publication, Rodgers et al. (*Science, (Washington, D.C.)* **1988**, *240*, 1675) published similar studies on the interaction of recombinant rat cytochrome b_5 with horse heart cytochrome *c*. Our results differ from theirs in one important respect: the changes in volume on complex formation that we observe are approximately 50% of the values they report. In the absence of a precise account of the methods employed by Rodgers et al., we temporarily ascribe this difference to our use of different proteins, recombinant rat cytochrome b_5 versus trypsin-solubilized bovine liver microsomal cytochrome b_5 and native cytochrome *c* versus porphyrin cytochrome *c*. We note that the recombinant rat cytochrome b_5 itself is reported to be sensitive to pressure and that the volume changes calculated by Rodgers et al. may include a contribution arising from heme solvation as well as from the cytochrome b_5-cytochrome *c* interaction.

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Total Synthesis of (+)-Didemnenones A and B. Absolute Configurations of the Didemnenones

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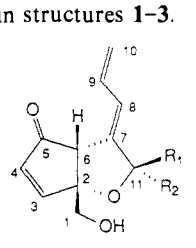
The didemnenones—recently discovered, densely functionalized, and biologically active—are marvelous synthetic targets.[1] We have recently completed an efficient enantiospecific total synthesis of didemnenones A (**1**) and B (**2**) and established the absolute configurations shown in structures **1-3**.

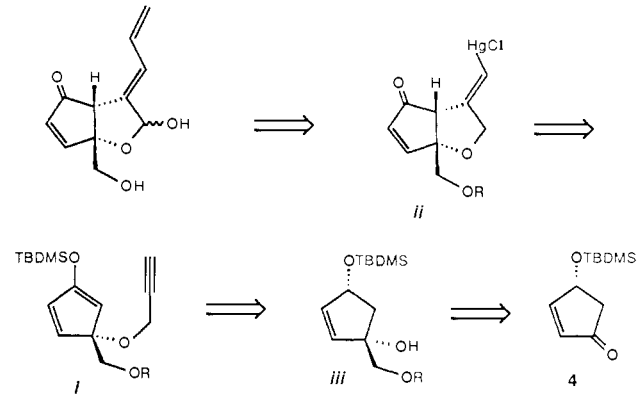


1 R_1 = H, R_2 = OH
2 R_1 = OH, R_2 = H
3 R_1 = H, R_2 = OCH_3

The Caribbean tunicate *Trididemnum* cf. *cyanophorum* produced didemnenones A (**1**) and B (**2**), while halfway around the world, the South Pacific tunicate *Didemnum voeltzkowi* produced didemnenones C and D.[1] The relative stereostructures of the didemnenones were established by an X-ray diffraction analysis of the acetal **3** derived from **1** and **2** followed by chemical and spectral correlations.[1]

Our approach to didemnenones A (**1**) and B (**2**) is shown in Scheme I. The most difficult issues were forming the C6-C7 bond and controlling the stereochemistry at C8. The C6-C7 bond could be formed by using a mercuric chloride induced cyclization[2]

Scheme I



of acetylenic silyl enol ether **i**. This transformation would be followed by the stereospecific conversion of the resultant vinyl mercurial **ii** to the corresponding halide and formation of the C8-C9 bond by using the recently reported palladium-catalyzed coupling of alkenyl halides and vinyl tin reagents.[3] Since replacement of the mercury and formation of the C8-C9 bond were both expected to proceed with retention, the *E*-diene would be formed.[3,4] Allylic oxidation would provide the proper oxidation level at C11. The configuration at C6 in the cis-fused cyclization product **ii** would be defined by the configuration at C2 in cyclization precursor **i**. The configuration at C2, in turn, could be established in **iii** via 1,3-chirality transfer in a diastereofacial selective nucleophilic addition to the chiral enone **4**.[5] Since both antipodes of **4** were available,[6] both enantiomers of **1** and **2** could be prepared.

The synthesis began with the addition of the hydroxymethyl anion equivalent *tert*-butoxymethyllithium[7] (1.4 equiv in tetrahydrofuran(THF)/*tert*-butyl methyl ether, -78 °C, 5 min) to (*R*)-4-(*tert*-butyldimethylsilyloxy)-2-cyclopentenone (**4**)[8] to afford (1*S*,4*R*)-1-(*tert*-butoxymethyl)-4-(*tert*-butyldimethylsilyloxy)-2-cyclopentenol (**5**)[9] (74.5% yield, $[\alpha]_D^{21}$ + 79.6° (*c* 0.950, $CHCl_3$)). The desired (1*S*,4*R*) adduct was readily separated from the (1*R*,4*R*) adduct (7:1 ratio of diastereomers) by silica gel chromatography (hexane-ethyl acetate, 4:1). The stereochemistry of the individual isomers of **5** was established by ^1H NMR analysis (see Supplementary Material). As anticipated, nucleophilic attack occured predominantly from the face trans to the silyloxy group of **4** and established the key stereocenter in **5**. Ether formation (propargyl bromide, NaH, THF, 96.0%) gave **6**, which was desilylated (nBu$_4$NF, THF, 96.2%) to **7** and oxidized to enone **8** (pyridinium dichromate, CH_2Cl_2, 94.3%). Silyl enol ether for-

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(8) (*R*)-**4** ($[\alpha]_D^{21}$ +62.8° (*c* 2.50 g/100 mL, MeOH, ca. 94% ee) (lit.[6a] $[\alpha]_D^{22}$ +66.6° (*c* 1.0, MeOH) for enantiomerically pure (*R*)-**4**)) was prepared by silylation (TBDMSCl, $(i\text{Pr})_2(\text{Et})\text{N}$, DMAP, CH_2Cl_2) of (*R*)-4-hydroxy-2-cyclopentenone obtained from (-)-(2*S*,3*S*)-tartaric acid according to ref 6g. (*S*)-**4** ($[\alpha]_D^{24}$ -43.6° (*c* 0.184 g/100 mL, MeOH, ca. 65% ee)) was similarly prepared from (+)-(2*R*,3*R*)-tartaric acid.

(9) All reactions involving air-sensitive compounds were performed under argon or nitrogen. All new compounds gave satisfactory spectral and analytical data, which are included in the supplementary material.

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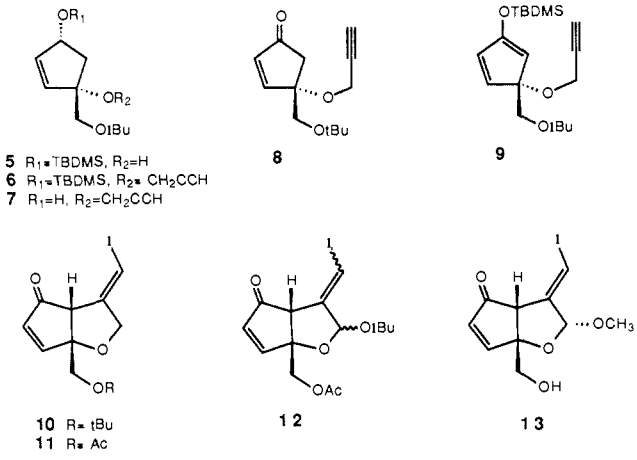
mation (*tert*-butyldimethylsilyl trifluoromethanesulfonate, triethylamine (Et_3N), CH_2Cl_2, 0 °C to 23 °C, 1 h)[10] coupled with a non-aqueous workup gave the cyclopentadiene **9**, which was submitted directly to mercuric chloride cyclization[2] (1.0 equiv of $HgCl_2$, 0.2 equiv of hexamethyldisilazane, CH_2Cl_2, 30 °C, 70 min). The cyclized vinyl mercurial was subjected without isolation to iodine mediated electrophilic substitution[4,11] (1.0 equiv of *N*-iodosuccinimide, 2 equiv of NaI, 0 to 23 °C, 4 h) to give, after workup and chromatography, the crystalline *E*-vinyl iodide **10**[12] (90.6% from **8**, mp 94-96 °C).



5 R_1=TBDMS, R_2=H
6 R_1=TBDMS, R_2= CH_2CCH
7 R_1=H, R_2=CH_2CCH

8 **9**

10 R= tBu
11 R= Ac

12 **13**

With the successful formation of **10**, three tasks remained to complete the synthesis: vinyl coupling, allylic oxidation, and unmasking of the primary alcohol. Treatment of **10** with acetic anhydride–ferric chloride[13] (0 °C, 1 h) provided acetate **11** (88.0%, mp 63 °C). Selenium dioxide oxidation[14] of **11** (0.7 equiv of SeO_2, 4.0 equiv of tBuOOH, 1,2-dichloroethane, 83 °C, 8 h) yielded an *E*,*Z* mixture of *tert*-butoxy acetal anomers **12** (81% combined yield, *E*:*Z* ca. 1:1), which, after methanolysis (catalytic TsOH, MeOH, 21-23 °C, 4 days) and chromatography, gave methoxy acetal **13** (24% yield from **11**) as the major *E*-vinyl iodide anomer. Coupling of **13** with tri-*n*-butylvinylstannane (catalytic $(Ph_3P)_2PdCl_2$, *N*,*N*-dimethylformamide, 23-24 °C, 18 h)[3] gave stereospecifically the (*E*)-diene **3** (72%, mp 127-128 °C, $[\alpha]_D^{21}$ +346.9° (*c* 0.179, $CHCl_3$)) which matched (^1H NMR, ^{13}C NMR, IR, UV, EIMS, TLC, mp, sign of specific rotation, and unit cell constants) acetal **3** derived from naturally occurring didemnenones A (**1**) and B (**2**). The acetal enriched in the opposite enantiomer[15] was obtained from (*S*)-**4**[8] by the same route. Hemiacetals **1** and **2** (1:1 mixture, $[\alpha]_D^{22}$ +514.8° (*c* 0.081, DMSO)) were obtained by hydrolysis of **3** (catalytic HCl, THF-H_2O, 2:1, 0-23 °C, 2.75 h, 69.9%) and were indistinguishable from the natural product mixture by ^1H NMR, IR, UV, EIMS, TLC, and sign of specific rotation.

The excess enantiomers of synthetic **1**, **2**, and (+)-**3** have the same absolute configurations as their naturally derived counterparts.[16] The absolute configurations at C2 and C6 are defined by the synthesis as 2*R*,6*R* for **1**, **2**, and (+)-**3**. On the basis of the structural correlations reported in our earlier work,[1] it follows that didemnenone C is 2*S*,6*S* and didemnenone D, most plausibly, is 2*S*,6*R*.

Acknowledgment. We thank NIH Grant CA24487, the New York State Sea Grant, and an NIH Training Grant for partial support of this work.

Supplementary Material Available: Analytical data for **1-3** and **5-13** (5 pages). Ordering information is given on any current masthead page.

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(11) Riediker, M.; Schwartz, J. *J. Am. Chem. Soc.* **1982**, *104*, 5842-5844.
(12) While subsequent allylic oxidation yields an *E*,*Z* mixture of vinyl iodide isomers, assignment of the *E* configuration to **10** is supported by protonolysis of the vinyl mercurial and assignment of the ^1H NMR resonances of the resulting exocyclic methylene protons as described in ref 3, as well as by vinyl coupling to form the corresponding *E*-diene as for **13**.
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(16) The specific rotation of the natural products **1** and **2** (1:1 mixture) is $[\alpha]_D$ +576.1° (*c* 0.49, DMSO), while that for naturally derived **3** is $[\alpha]_D$ +371.8° (*c* 0.86, $CHCl_3$).[1] The estimated optical purities of synthetic **1**, **2**, and (+)-**3** (ca. 89-93% ee) approximate that estimated for starting material (*R*)-**4** (ca. 94% ee).[8]

Origin of the Cyanamide Carbon of the Kinamycin Antibiotics†

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We have previously described the biosynthesis of the benz[*b*]carbazole skeleton of the kinamycin antibiotics **1**-**4**[2-5] from acetate via the benz[*a*]anthraquinone **5** (Scheme I).[6] The data suggested excision from **5** of two carbons, C-5 and C-6, originally derived from the same acetate unit. The only primary metabolic precursor of kinamycin remaining to be identified was that of the cyanamide unit. Efforts to identify the origin of this unusual moiety[7] were hampered by the apparent absence of its resonance signal from the ^{13}C NMR spectra of the kinamycins.[8] We now report experiments that uncovered the missing resonance as well as experiments that reveal the source of the cyano carbon.

Fermentation of *Streptomyces murayamaensis* in a defined medium[10] containing $(^{15}NH_4)_2SO_4$ as the sole nitrogen source afforded [^{15}N]kinamycin D, **4a**. The 40.5 MHz ^{15}N NMR spectrum (Figure 1) showed two doublets (J_{NCN} = 3.4 Hz)[11] at 344.5 and 241.6 ppm relative to $H^{15}NO_3$ at 362.0 ppm, for the two nitrogens. The 100.6 MHz ^{13}C NMR spectrum of **4a** (Figure 2) showed ^{15}N-coupled doublets for C-5′ (δ 131.7, J_{CN} = 2.9 Hz), C-6′ (δ 128.0, J_{CN} = 2.2 Hz), C-2 (δ 132.2, J_{CN} = 2.3 Hz), and C-3 (δ 128.4, J_{CN} = 2.8 Hz). To our surprise and delight a doublet of doublets was also observed at δ 78.5, J_{CN} = 21.2, 5.4 Hz. Examination of all previously obtained ^{13}C NMR spectra of **4** revealed the small singlet, almost overlapped by the $CDCl_3$ resonance, that had been ignored as an impurity. Although we cannot unequivocally explain the large upfield shift (ca. 30 ppm),[8] its observation has allowed us to determine the biosynthetic origin of the cyanamide carbon.

Feedings with typical one-carbon precursors labeled with ^{14}C led only to low incorporations ([$^{14}CH_3$]methionine, 0.08%; sodium [^{14}C]formate, 0.01%; sodium [^{14}C]cyanide, 0.01%; [^{14}C]urea, 0.01%; sodium [^{14}C]carbonate, 0.05%) except for [guanido-^{14}C]arginine, (0.61%),[12] [3-^{14}C]serine (0.49 and 1.90%[13]), and

† Dedicated to Professor Duilio Arigoni on the occasion of his 60th birthday.
(1) Career Development Awardee of the National Cancer Institute (Grant CA-00880), 1979-1984.
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