ESR Spectroscopy. A Bruker 200D SRC instrument was used to obtain X-band ESR spectra. Temperatures in the range of 300 to 100 K were controlled by a nitrogen flow system equipped with heat exchanger, Pt-resistance thermometer, and heater (Varian); temperatures were measured using a thermocouple. Half-field, $\Delta m_s = 2$, transition was detected.

UV–Vis Spectroscopy. UV–vis absorption spectra were recorded at ambient temperature in a 2 mm-pathlength quartz cell using a Perkin-Elmer Lambda 6 spectrophotometer. The spectrometer sample chamber was accessible from a glovebox. Preparations of all solutions were carried out in a glovebox under an argon atmosphere.

A solution of $2^{2-},2Li^+$ in THF was obtained under similar conditions as in the preparation of the NMR sample of $2^{2-},2Li^+$ in THF-d_8. Small aliquots from the reaction mixture were taken and diluted with 7×10^{-4} M MeLi/THF before recording the spectrum. For a reaction mixture, which was 0.7 M in MeLi and 0.1 M in 2-$(H)_2$, UV–vis spectra recorded after both 16 and 22 h at ambient temperature showed $\lambda_{max} = 463$ nm.

The reaction of 2-$(H)_2$ (3×10^{-5} M) with MeLi (0.02 M) in THF was also followed directly with UV–vis spectroscopy. The spectra were acquired at 10-min intervals and, after several hours, at 1-h intervals. A steady rise of the $\lambda_{max} = 463$ nm band was observed.

Reaction of $6^{2\bullet}$ with Li in THF was followed with UV–vis spectroscopy as already described for similar systems.[6a]

Electrochemistry. All electrochemical measurements were carried out in a glovebox using a PARC Model 270 electrochemistry system as described previously.[7] THF was used as solvent. The concentration of the electroactive solute was 0.001–0.003 M and of supporting electrolyte, tetrabutylammonium perchlorate (TBAP), was 0.1–0.2 M. Three-electrode home-made voltammetric cells were used: silver wire quasi-reference electrode, Pt foil counter electrode, and a Pt disk working microelectrode (dia. 100 μm, BAS). The solution volume was 2 mL. Ferrocene (0.510 V vs SCE) was used as a reference.[21] Separation between potential at the peaks of the oxidation and reduction CV waves was about 100 mV for ferrocene and diradical.

SQUID. Magnetic measurements were carried out on two SQUID magnetometers (MPMS, Quantum Design) at the University of Nebraska—Lincoln and at NIST in Boulder, CO. The technical details of measurements and sample preparation were described previously. The measurements at NIST were carried out by Robert Loughran.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for 24 closed-shell molecules and NMR spectra for carbodianions (25 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(21) Ferrocene. Diggle, J. W.; Parker, A. J. *J. Electrochem. Acta* **1973**, *18*, 976.

Polydiscamide A: A New Bioactive Depsipeptide from the Marine Sponge *Discodermia* sp.

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A depsipeptide, polydiscamide A (1), composed of 13 amino acids including a novel amino acid 3-methylisoleucine, was isolated from a Caribbean sponge, *Discodermia* sp. Its structure was elucidated by means of spectroscopic, chemical degradation, and derivatization techniques. Polydiscamide A inhibits the in vitro proliferation of the cultured human lung cancer A549 cell line.

Marine sponges are a well-established source of unique and of biologically active peptides. Discodermins A–D[1-3] from *Discodermia kiiensis*, theonellapeptolide F[4] from *Theonella* sp., jaspamide[5,6] from *Jaspis* sp., fenistins[7] from *Leucophloeus fenestrata*, and geodiamalide[8] from *Pseudaxinyssa* sp. are recent examples. The occurrence of biologically active novel structural types calyculins,[9] discodermolide,[10] and discodermide[11] in several *Discodermia* spp. led us to extend our research on a related undescribed species of deep-water sponge of the genus *Discodermia*.

(1) Matsunaga, S.; Fusetani, N.; Konosu, S. *J. Nat. Prod.* **1985**, *48*, 236.
(2) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, *25*, 5165.
(3) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron. Lett.* **1985**, *26*, 855.
(4) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Walchli, M. *J. Am. Chem. Soc.* **1989**, *111*, 2582.
(5) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123.
(6) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797.
(7) Omar, S.; Tenenbaum, L.; Manes, L. V.; Crews, P. *Tetrahedron Lett.* **1988**, *29*, 5489.
(8) de Silva, E. D.; Andersen, R. J.; Allen, T. M. *Tetrahedron Lett.* **1990**, *31*, 489.
(9) (a) Kato, Y.; Fusetanii, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780. (b) Kato, Y.; Fusetanii, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930. (c) Matsunaga, S.; Fujiki, H.; Sakata, D. *Tetrahedron* **1991**, *47*, 2999.
(10) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912.
(11) Gunasekera, S. P.; Gunasekera, M.; McCarthy, P. *J. Org. Chem.* **1991**, *56*, 4830.

0022-3263/92/1957-1767$03.00/0

Table I. ^{13}C and ^{1}H NMR Data for Polydiscamide A (1)[a]

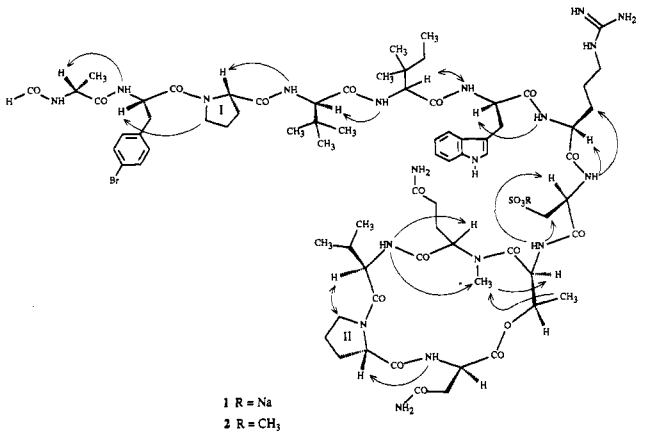
	DMSO-d_6/TFA		CD_3OD	
	^{13}C	^{1}H	^{13}C	^{1}H
Ala				
α	46.52 (d)	4.31 (m)	48.87	4.35 (q, 7.2)
β	18.49 (q)	0.94 (d, 7.0)	18.49	1.11 (d, 7.1)
NCHO	160.61 (d)	7.90 (s)	163.40	8.00 (s)
NH	–	8.12 (d, 7.8)	–	–
BrPhe				
α	51.53 (d)	4.70 (ddd, 10.5, 8.5, 2.3)	53.55	4.85 (dd, 4.4, 9.7)
β	36.34 (t)	2.70 (dd, 13.8, 10.5)	37.76	2.81 (dd, 9.7, 14.2)
		2.97 (dd, 13.8, 2.3)		3.12 (dd, 4.4, 14.2)
C4	136.16 (s)	–	137.72	–
C5/C9	131.68 (d)	7.21 (2 H, d, 8.3)	132.66	7.16 (2 H, d, 8.4)
C6/C8	130.80 (d)	7.39 (2 H, d, 8.3)	132.46	7.40 (2 H, d, 8.4)
C7	119.49 (s)	–	121.55	–
NH	–	8.33 (d, 8.5)	–	–
Pro I				
α	60.55 (d)	4.54	61.65	4.60 (dd, 4.0, 8.2)
β	29.51[b] (t)	2.12 (m), 1.89 (m)	30.92	2.25 (m), 2.07 (m)
γ	24.16 (t)	1.88 (m)	25.66	1.99 (m)
δ	46.95 (t)	3.62 (2 H, m)	48.62	3.74 (m), 3.61 (m)
t-Leu				
α	59.46 (d)	4.51 (d, 10)	61.56	4.49 (s)
β	35.12 (s)	–	36.56	–
γ	26.72 (q)	0.89 (9 H, s)	27.50	1.01 (9 H, s)
NH	–	7.70 (d, 10)	–	
3-MeIle				
α	59.75[c] (d)	4.13 (d, 7.5)	62.75	4.09 (s)
β	35.86 (s)	–	36.90	–
γ	31.12 (t)	1.06 (m)	32.77	1.12 (m)
δ	7.89 (q)	0.62 (t, 7.2)	8.35	0.69 (t, 7.3)
γ′	22.71 (q)	0.62 (s)	23.27	0.67 (s)
γ″	22.99 (q)	0.73 (s)	23.60	0.80 (s)
NH	–	7.81 (d, 7.5)	–	–
Trp				
α	53.71 (d)	4.61 (m)	55.81	4.74 (m)
β	27.58 (t)	3.16 (dd, 5, 14)	28.35	4.41 (dd, 6.0, 14.8)
		2.92 (m)		3.12 (m)
C4	109.87 (s)	–	111.32	–
C5	124.02 (d)	7.13 (s)	124.98	7.15 (s)
C6	137.04 (s)	–	138.18	–
C7	127.12 (s)	–	128.62	–
C8	118.32 (d)	7.56 (d, 7.8)	119.46	7.57 (d, 7.7)
C9	118.14 (d)	6.94 (t, 7.4)	119.83	6.98 (dd, 7.4, 7.7)
C10	120.77 (d)	7.02 (t, 7.4)	122.44	7.07 (dd, 7.3, 8.1)
C11	111.22 (d)	7.31 (d, 8.1)	112.33	7.32 (d, 8.1)
NH	–	8.17 (d, 7.3)	–	–
NH-aromatic		10.63 (br d s)	–	–
Arg				
α	52.19 (d)	4.31 (m)	54.72	4.39 (m)
β	28.83 (t)	1.61 (br, m)	30.07	1.83 (m), 1.67 (m)
γ	23.60 (t)	1.32 (br, m)	25.53	1.49 (m), 1.41 (m)
δ	40.26 (t)	2.99 (m)	41.90	3.09 (m)
guan	156.76 (s)	–	158.53	–
NH	–	8.00 (d, 7.3)	–	–
cysteic acid				
α	50.81 (d)	4.63 (m)	52.68	4.79 (dd, 4.2, 9.2)
β	52.19 (t)	2.95 (m)	52.01	3.26 (dd, 4.2, 14.1)
				3.34 (dd, 9.2, 14.1)
NH	–	8.21 (d, 7.4)	–	–
Thr				
α	51.89 (d)	4.91 (br d, 10.5)	54.38	4.99 (br d, 1.2)
β	69.93 (d)	5.10 (m)	70.37	5.43 (m)
γ	15.91 (q)	1.17 (d, 6.3)	17.32	1.27 (d, 6.3)
NH	–	7.91 (d, 8.2)	–	–
NMeGln				
α	54.94 (d)	5.10 (m)	57.01	5.11 (m)
β	23.60 (t)	1.99 (m), 1.89 (m)	25.10	2.16 (m)
γ	31.67 (t)	1.8–2.2	32.61	1.9–2.3
NMe	30.43 (q)	3.02 (s)	31.63	3.15 (s)
Val				
α	55.24 (d)	4.38 (t, 7.8)	57.62	4.48 (d, 8.1)
β	30.00 (d)	1.94 (m)	31.94	2.05 (m)
γ	19.24 (q)	0.85 (d, 6.8)	19.50	0.96 (d, 6.2)
δ	17.90 (q)	0.82 (d, 6.6)	18.85	0.94 (d, 5.5)
NH	–	7.67 (d, 7.5)	–	–

Table I (Continued)

	DMSO-d_6/TFA		CD_3OD	
	^{13}C	^1H	^{13}C	^1H
Pro II				
α	59.89[c] (d)	4.20 (br d, 7.2)	62.53	4.39 (m)
β	29.70[b] (t)	2.00 (m), 1.92 (m)	31.17	2.18 (m)
γ	24.23 (t)	2.00 (m), 1.92 (m)	25.75	2.25 (m), 1.99 (m)
δ	46.86 (t)	3.88 (m), 3.52 (m)	48.87	4.05 (m), 3.68 (m)
Asn				
α	48.26 (d)	4.55 (m)	not observed	4.64 (m)
β	35.12 (t)	2.46 (br d, 16.1)	36.22	2.55 (br d, 15.20)
		2.83 (dd, 7.6, 16.1)		2.98 (br d, 17.5)
NH	–	7.28 (d, 7.6)	–	–

[a] Chemical shift δ (multiplicity, coupling constant in hertz). [b] May interchange. [c] May interchange. Carbonyl signals: (in DMSO-d_6) δ 173.58, 171.86, 171.48, 171.15, 171.05, 170.80, 170.75, 170.55, 170.40 (2 C), 170.32, 170.24, 169.74, 169.48, 168.85; (in CD_3OD) δ 177.34, 174.0 (3 C), 173.75 (2 C), 173.23, 173.14, 173.02, 172.90, 172.71, 172.65, 172.25, 171.46, 171.30.

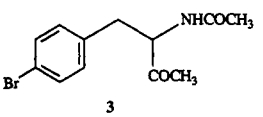
We now report the isolation and structure determination of a new depsipeptide, polydiscamide A (1). Polydiscamide A inhibits the in vitro proliferation of the cultured human lung cancer A549 cell line[12] (IC_{50} 0.7 μg/mL). It also inhibits the growth of *Bacillus subtilis* (MIC of 3.1 μg/mL).



1 R = Na
2 R = CH_3

The MeOH extract of the sponge *Discodermia* sp. was concentrated to a syrup and partitioned between equal volumes of water and EtOAc. The water-soluble fraction on reversed-phase column chromatography followed by reversed-phase HPLC [MeOH–water (80:20) containing acetic acid (2.2 mL/L)] yielded pure polydiscamide A. The molecular formula of 1 was determined to be $C_{75}H_{109}{}^{81}BrN_{19}O_{20}SNa$ (HRFABMS M + 2H at m/z 1733.7045, Δ 1.4 mmu). Amino acid analysis by reversed-phase HPLC of the phenyl isothiocyanate derivatives[13,14] confirmed 10 of the 13 amino acids: alanine, two prolines, *tert*-leucine, tryptophan, arginine, cysteic acid, threonine, valine, and aspartic acid. Analysis of ^1H and ^{13}C NMR data (Table I) and ^1H–^1H COSY, ^1H–^1H LRCOSY, ^1H–^1H COSYRCT,[15] DEPT,[16] HETCOR,[17] and HMBC[18] data revealed the presence of *p*-bromophenylalanine, 3-methylisoleucine, and *N*-methylglutamine in addition to the amino acids established by amino acid analysis. The UV absorption spectrum (MeOH) of 1 gave λ_{max} nm (ϵ) 214 (44900), 272 (5300), 280 (5400), 288 (4600) which was consistent with the presence of bromophenylalanine and tryptophan residues in 1. Acid hydrolysis of 1 with 6 N HCl and derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) followed by reversed-phase HPLC comparison with standards[19] furnished the stereochemistry of the above 10 amino acids and *p*-bromophenylalanine. A new amino acid in 1, 3-methylisoleucine, was identified by ^1H NMR signals (DMSO-d_6/TFA) at δ 4.13 (d, J = 7.5 Hz, αH), 1.06 (m, γCH_2), 0.62 (t, J = 7.2 Hz, δMe), 0.62 (s, γ'Me), and 0.73 (s, γ''Me) which indicated that it has an αH, an ethyl group, and two methyl groups. The relatively high field ^{13}C NMR chemical shift of the δ-methyl at δ 7.89 was consistent with the presence of a *gem*-dimethyl substitution[20] at the βC. The absence of protons on the β carbon was further confirmed by the appearance of the αH as a doublet in DMSO/TFA and a singlet in MeOH. Long range C–H correlations observed in an HMBC experiment (γ'H/αC, γC, and γ''H/αC, γC, γ'C; αH/γ'C and γH/βC) confirmed its structure.

Acid hydrolysis of polydiscamide A followed by methylation[21] with CH_2N_2 and subsequent acetylation with acetic anhydride/pyridine gave a mixture of derivatized amino acids and small peptide units. Purification by reversed-phase HPLC yielded *N*-acetyl-*p*-bromophenylalanine methyl ester (3), dimethyl *N*-acetylaspartate (4), *N,O*-diacetylthreonine methyl ester (5), and a dipeptide, *N*-acetyl-*tert*-leucyl-3-methylisoleucine methyl ester (6).



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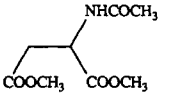

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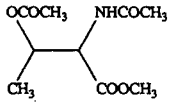

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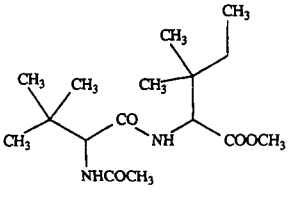

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The ^1H NMR, ^{13}C NMR, and HRMS data confirmed their

(12) Lieber, M.; Smith, B., Szakl, A.; Nelson-Rees, W.; Todaro, G. *Int. J. Cancer* **1976**, *17*, 62.
(13) Heinrikson, R. L.; Meredith, S. C. *Anal. Biochem.* **1984**, *136*, 65.
(14) Scholze, H. *J. Chromatogr.* **1985**, *350*, 453.
(15) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.
(16) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Williams, D. H. *J. Org. Chem.* **1982**, *47*, 3021.
(17) Bax, A.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 501.
(18) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(19) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
(20) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley and Sons: New York, 1988; p 113.
(21) Fales, H. M.; Jaouni, T. M.; Babashak, J. F. *Anal. Chem.* **1973**, *45*, 2302.

structures. The αH of *N*-Me-Gln at δ 5.10 was coupled to the β-CH_2 at δ 1.99 and 1.89. HMBC results indicated three-bond coupling between the *N*-Me protons and the αC. From irradiation of the αH in a difference decoupling experiment, the βHs were found to be reduced to multiplets, indicating the presence of adjacent γ-methylene protons. Absence of COSY correlations between the βHs and any signal outside the methylene envelope of δ 1.8–2.2 other than the corresponding αH at δ 5.10 suggested that the γHs corresponded to the resonances observed around δ 1.8–2.2. The ^{13}C NMR signals[22] at δ 54.94 (α-CH), 23.60 (β-CH_2), 31.67 (γ-CH_2), 30.43 (N-Me) further confirmed the presence of the *N*-methylglutamine unit in the molecule.

Reaction of polydiscamide A in MeOH with CH_2N_2 in ether yielded polydiscamide A methyl ester (**2**), which suggested the presence of an acid group in the molecule. The absence of significant chemical shift changes in the carbonyl region of the ^{13}C spectrum of polydiscamide A methyl ester when compared with that of polydiscamide A suggested that the sulfonic acid group of the cysteic acid was the only acid functionality in the molecule. These data combined with the ^1H and ^{13}C NMR data suggested that the aspartic acid and the *N*-methylglutamic acid units should exist as amides or esters. The low-field chemical shift values of the βH (δ 5.10) and the βC (δ 69.93) of threonine indicated that the oxygen atom at the βC is a part of a lactone ring system. The three-bond C–H correlation observed between alanine αC and the formamide proton in the HMBC experiment identified the alanine as the N-terminal amino acid of the peptide. The HMBC experiment also furnished the intra-amino acid connectivities in BrPhe, αC/βH; *t*-Leu, αC/γMe, βC/γMe, γC/αH; Try, αC/βH; Thr, αC/γMe, βC/γMe; Pro, αC/βH, αC/γH; Asn, βC/αH. However, due to the crowding of signals in the carbonyl region of the ^{13}C NMR spectrum, the HMBC data did not allow unambiguous assignment of the connectivities between the individual amino acids. The connectivities between the amino acid units as shown in **1** were established using 1D difference and 2D NOE[23] experiments. The following 1D NOE's (in DMSO-d_6/TFA) were observed [H(s) irradiated/H(s) enhanced (% negative enhancement)]: BrPhe NH/Ala αH (1%); Cyst NH/Arg α (2%), Cyst α (2%), Arg β (1%), Cyst β (0.6%); Trp NH/3MeIleu α (4%); Arg NH/Trp α (1%); Thr NH/Cyst α (2%); 3MeIleu NH/*t*-Leu α (3%), 3MeIleu α (1%); *t*-Leu NH/ProI α (2%); Val NH/NMeGln α (2%); Asn NH/Pro II α (1%), Pro II δ at 3.88 (0.5%), Asn β (1%); Val α/Pro II δ at 3.52 (4%), Pro II δ at 3.88 (3%), Val NH (2%); 3MeIleu α:Trp NH (4%); Pro II δ at 3.88:Pro δ at 3.52 (6%), Val α (4%); NMe of NMeGln:Thr α (8%); Thr Me:Thr NH (1%), Thr β (1%), NMe of NMeGln (0.3%); Thr β and NMeGln α (overlapped):Val NH (3%), NMe of NMe Gln (1%). NOESY experiments carried out in DMSO-d_6/TFA gave further evidence for the connectivity of the amino acid units. The correlations observed were Ala αH/BrPhe NH; BrPhe αH/Pro I δH; *t*-Leu NH/Pro I αH; *t*-Leu βMe/*t*-Leu αH; *t*-Leu αH/3MeIleu NH; 3MeIleu βMe/3MeIleu αH; 3MeIleu αH/TrpNH; Trp αH/Arg NH; Arg αH/cysteic acid NH; cysteic acid αH/Thr NH; Thr αH/NMe Gln Me; Thr βMe/NMe Gln Me; NMe Gln αH/Val αH; Val Me/Val αH; Val αH/Pro II δH; Pro II αH/Asn NH. These results established the peptide chain had the following sequence OHC-NH-Ala-BrPhe-Pro-*t*-Leu-βMeIleu-Trp-Arg-Cys-(O_3Na)-Thr-NMeGln-Val-Pro-Asn. The γH of the *N*-MeGln at δ 1.8-2.2 was consistent with the uncyclized amide side chain. Generally the ring formation with the Gln side chain shifts the α-protons to low-field chemical shift values.[24,25] This established the connectivity of the *N*-MeGln to the peptide chain via its C-1 carbonyl and the *N*-methyl amino group. This observation allowed the formation of the ester between Asn and the βOH of Thr. Comparison of the ^1H and ^{13}C chemical shift values of the α and β protons and carbons of Asn with the literature values[22] established the esterification at C-1 carbon. This established the amide group at C-4, which satisfied the molecular formula. Combination of the above data determined the structure of polydiscamide A.

Compared to discodermins, polydiscamide A contained *p*-bromophenylalanine instead of phenylalanine in the side chain. The novel amino acid 3-methylisoleucine in **1** replaced one of the *tert*-leucine of discodermin A [HCO-D-Ala-L-Phe-L-Pro-D-*t*-Leu-L-*t*-Leu-D-Trp-L-Arg-D-Cys-(O_3H)-L-Thr-L-MeGln-D-Leu-L-Asn-L-Thr-Sar]. The lactone ring of polydiscamide A consisted of five amino acids instead of six amino acids in discodermins. The leucine, threonine, and sarcosine in the ring portion of discodermins were replaced by valine and proline in polydiscamide A. Additionally, the common amino acids in both polydiscamide A and discodermins indicated the same absolute stereochemistry except for Asn, which had the D configuration in polydiscamide A.

Experimental Section

General Procedures. NMR spectra were recorded at 360 MHz for ^1H and 90.5 MHz for ^{13}C. All chemical shifts were recorded with respect to the residual proton signals of the deuterated solvents.

Collection and Extraction. The sponge *Discodermia* sp. (order Lithistida) was collected using the *Johnson Sea Link* submersible from St. Lucia, Lesser Antilles, at a depth of 274 m. The sponge consists of firm, hollow tubes (1–3-cm diameter) which branch from a central stalk. The branches may have a single, apical oscule. This undescribed species of *Discodermia* was originally field-identified as *D. polydiscus*, but was subsequently found to be an undescribed species. It was similar to *D. polydiscus* in consistency and chemistry. Both species are hard in consistency as a results of skeletal architecture of the spicules: the desmas are arranged in an intricate, interconnecting network in *D. polydiscus* and less so in *Discodermia* sp. Polydiscamide A was found in samples of both species. A taxonomic voucher specimen of the sample of *Discodermia* sp. from which the polydiscamide A was isolated is deposited at the Harbor Branch Oceanographic Museum (catalog number 003-00145).

Isolation of Polydiscamide A (1). *Discodermia* sp. (1107 g of wet sponge) was soaked in MeOH (2 L) overnight, ground in a blender, and filtered. The residue was successively extracted with MeOH (1 L), MeOH/EtOAc (500 mL/500 mL), and EtOAc (2 × 1 L). The MeOH extracts were combined, concentrated to a gum (35 g), and partitioned between EtOAc and water (4 × 500 mL; 500 mL). The aqueous layer was concentrated to dryness, triturated with MeOH, and filtered. The filtrate was concentrated to dryness (14.1 g). A portion (5.5 g) was dissolved in MeOH/water (1:1) and chromatographed on a reversed-phase C_{18} column under vacuum with MeOH/water (1:1), followed by MeOH/water (8:2) and MeOH. The fraction that eluted with MeOH/water (8:2) was concentrated to dryness (600 mg) and rechromatographed on a RP-C_{18} cartridge with MeOH. This fraction was concentrated to dryness and purified by HPLC on Dynamax Macro reversed-

(22) Moore, R. E.; Bornemann, V.; Niemczura, W. P.; Gregson, J. M.; Chen, J.-L.; Patterson, G. M. L.; Helms, G. L. H. *J. Am. Chem. Soc.* **1989**, *111*, 6128.

(23) Edwards, M. W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918.

(24) Namikoshi, M.; Rinehart, K. L.; Sakai, R.; Sivonen, K.; Carmichael, W. W. *J. Org. Chem.* **1990**, *55*, 6135.

(25) Harada, K.-I.; Ogawa, K.; Matsuura, K.; Nagai, H.; Murata, H.; Suzuki, M.; Itezono, Y.; Nakayama, N.; Shirai, M.; Nakano, M. *Toxicon* **1991**, *29*, 479.

phase C_{18} column with MeOH/water (80/20) containing acetic acid (2.2 mL/L of solution) to yield polydiscamide A (188 mg, 0.05% of wet weight). Polydiscamide A (1): white powder; mp 212–216 °C; $[\alpha]^{24}_D$ – 1.1 (c = 1.89, MeOH): UV λ_{max} nm (ϵ) 214 (44 900), 272 (5300), 280 (5400), 288 (4600); IR (KBr) 3300 (br), 1754 (sh), 1704 (sh), 1614 (br) cm^{-1}; ^1H and ^{13}C NMR Table 1; LRFABMS m/z (% relative intensity) 1733 (17.1), 1731 (16.7), 1071 (6.7), 1047 (4.8).

Methylation of Polydiscamide A (1). Polydiscamide A (6 mg) in MeOH (0.5 mL) was treated with an excess CH_2N_2 in ether at rt for 1 h. The usual workup followed by purification on HPLC (reversed-phase C_{18}, 5 μm, 10- × 250-mm Altex column) with H_2O/MeOH (25:75) containing HOAc (2.2 mL/L solution) yielded polydiscamide A methyl ester (2) (3.8 mg): HRFABMS m/z 1718.7161, Δ 0 mmu for $C_{75}H_{112}N_{19}O_{19}S^{81}BrNa$ 1718.7161 (M – CO + Na)$^+$; ^1H NMR (CD_3OD) δ Ala: 4.35 (q, J = 7.1 Hz, αH), 1.11 (d, J = 7.1 Hz, βMe); BrPhe: 4.68 (dd, J = 9.6, 3.4 Hz, αH), 2.81 (dd, J = 9.6, 14.2 Hz, βH), 7.17 (d, J = 8.2 Hz, H-5/H-9), 7.40 (d, J = 8.2 Hz, H-6/H-8); Pro I: 4.60 (dd, J = 3.9, 8.2 Hz, αH), 2.26 (m, βH), 2.08 (m, βH), ~2.0 (m, γH), 3.75 (m, δH), 3.60 (m, δH); t-Leu: 4.51 (s, αH), 1.00 (9 H, s, δ Me); 3-MeIleu: 4.05 (s, αH), 1.11 (m, γH), 0.79 (s, Me), 0.68 (t, J = 7.4 Hz, Me), 0.65 (s, Me); Trp: 4.74 (m, αH), 3.42 (dd, J = 5.6, 15.0 Hz, βH), 3.10 (m, βH), 7.14 (s, H-5), 7.57 (d, J = 7.8 Hz, H-9), 6.99 (dd, J = 7.3, 7.8 Hz, H-10), 7.07 (dd, J = 7.3, 8.1 Hz, H-11), 7.32 (d, J = 8.1 Hz, H-12); Arg: 4.43 (m, αH), 1.85 (m, βH), 1.67 (m, βH), 1.48 (m, γH), 3.09 (m, δH); Cyst: 4.83 (m, αH), 3.31 (m, βH); Thr: 5.01 (br s, αH), 5.42 (br d, J = 6.9 Hz, βH), 1.28 (d, J = 6.7 Hz, γMe); NMeGln: 5.07 (m, αH), 2.17 (m, βH), 1.9–2.3 (m, γH), 3.12 (s, N-Me); Val: 4.40 (m, αH), 2.07 (m, βH), 0.98 (d, J = 6.8 Hz, γMe), 0.95 (d, J = 6.8 Hz, γMe); Pro II: 4.40 (m, αH), 2.2 (m, βH), 2.2 (m, γH), 2.02 (m, γH), 4.05 (m, δH), 3.67 (m, δH); Asn: 4.73 (m, αH), 2.61 (dd, J = 3.1, 16.9 Hz, βH), 3.03 (dd, J = 6.1, 16.9 Hz, βH); ^{13}C NMR (CD_3OD) δ 177.38 (s), 174.19 (s), 174.01 (s), 173.96 (s), 173.75 (2C, s), 173.28 (2 C, s), 172.93 (s), 172.67 (s), 172.34 (2 C, s), 172.14 (s), 171.49 (s), 171.35 (s), 163.37 (d), 158.57 (s), 138.20 (s), 137.74 (s), 132.65 (2 C, d), 132.48 (2 C, d), 128.58 (s), 124.94 (d), 122.45 (d), 121.59 (s), 119.82 (d), 119.43 (d), 112.33 (d), 111.39 (s), 70.73 (d), 62.88 (d), 62.36 (d), 61.65 (d), 61.50 (d), 57.93 (d), 57.44 (d), 55.73 (d), 54.70 (d), 53.56 (d), 53.08 (q), 52.67 (d), 52.14 (t), 49.98 (d), 48.79 (t), 48.64 (t), 41.93 (t), 37.77 (t), 36.87 (s), 36.60 (s), 35.91 (t), 32.78 (t), 32.70 (t), 31.75 (d), 31.75 (q), 31.24 (t), 31.01 (t), 30.71 (t), 30.16 (t), 28.40 (t), 27.52 (3 C, q), 25.78 (t), 25.57 (t), 25.50 (t), 25.08 (t), 23.58 (q), 23.21 (q), 19.48 (q), 18.98 (q), 18.49 (q), 17.31 (q), 8.35 (q).

Hydrolysis of 1. Polydiscamide A (1) (60 mg) was heated in 6 N HCl at 110 °C for 16 h. The hydrolyzate was dried by passing a stream of N_2, and the residue was dissolved in MeOH (50 mL) and reacted with excess CH_2N_2 in ether. The solvent was evaporated, and the residue was acetylated with Ac_2O (2 mL) and pyridine (0.5 mL) at rt for 25 h. The solvent was removed, and the residue was chromatographed on a silica cartridge (Supelco) with MeOH/CH_2Cl_2 (1:9, 10 mL). The eluate was concentrated to dryness (26.4 mg), redissolved in MeOH, and chromatographed on a reversed-phase C_{18} cartridge (Supelco) with MeOH as solvent. The eluate was concentrated to dryness (22 mg), and the residue was chromatographed on reversed-phase C_{18} HPLC (5 μm, 4.6 mm × 150 mm) with MeOH/H_2O (3:2). The fractions obtained were rechromatographed on the same column with MeOH/H_2O (2:3) to yield 3 (3.7 mg) and 6 (1.4 mg) and with MeOH/H_2O (1:9) to yield 4 (0.3 mg) and 5 (0.6 mg).

***N*-Acetyl-*p*-bromophenylalanine methyl ester (3):** ^1H NMR (CD_3OD) δ 7.43 (2 H, d, J = 8.4 Hz, H-6/H-8), 7.12 (2 H, d, J = 8.4 Hz, H-5/H-9), 4.65 (1 H, dd, J = 5.7, 8.9 Hz, αH), 3.68 (3 H, s, $COOCH_3$), 3.11 (1 H, dd, J = 5.7, 14.0 Hz, βH), 2.91 (1 H, dd, J = 8.9, 14.0 Hz, βH), 1.90 (3 H, s, $NHCOCH_3$); ^{13}C NMR (CD_3OD) δ 173.3 (s)/173.1 (s), $COOCH_3$/$NHCOCH_3$), 137.6 (s, C-4), 132.5 (2 C, d, C-5/C-9), 132.2 (2 C, d, C-6, C-8), 121.7 (s, C-7), 55.0 (d, αC), 52.7 (q, OMe), 37.8 (t, βC), 22.2 (q, $NHCOCH_3$); LRCIMS (isobutane) m/z (% relative intensity) 302 (M + H, 23.6), 300 (M + H, 25.7), 258 (5.5), 256 (5.9), 242 (M^+ – CH_3CONH_2, 18.6), 240 (M^+ – CH_3CONH_2, 19.9); HREIMS m/z 299.0138, Δ 1.9 mmu for $C_{12}H_{14}NO_3{}^{79}Br$ (M^+).

Dimethyl *N*-acetylaspartate (4): ^1H NMR ($CDCl_3$) δ 6.4 (1 H, br d, J = 8.0 Hz, NH), 4.84 (1 H, t, d, J = 4.5, 8.0 Hz, αH), 3.75/3.68 (2 × 3 H, s, 2 $COOCH_3$), 3.01 (1 H, dd, J = 4.5, 17.2 Hz, βH), 2.84 (1 H, dd, J = 4.5, 17.2 Hz, βH), 2.01 (3 H, s, $NCOCH_3$); ^{13}C NMR ($CDCl_3$) δ 52.8–51.6 (2 q, $COOCH_3$), 48.6 (d, αC), 36.2 (t, βC), 23.1 (q, $NCOCH_3$), 171.6, 171.2, 169.8 (3 s, 2 $COOCH_3$/$NCOCH_3$); HRCIMS (isobutane) m/z 218.1047, Δ 2.5 mmu for $C_9H_{16}NO_5$ (M + H)$^+$; LREIMS m/z (% relative intensity) 144 (14.0), 129 (33.0), 112 (13.5), 102 (23.6), 70 (22.1).

***N*,*O*-Diacetylthreonine methyl ester (5):** ^1H NMR ($CDCl_3$) δ 6.03 (1 H, br d, J = 9.4 Hz, NH), 5.40 (1 H, dq, J = 2.8, 6.6 Hz, βH), 4.77 (1 H, dd, J = 2.8, 9.4 Hz, αH), 3.72 (3 H, s, $COOCH_3$), 2.08 and 2.01 (6 H, s, $OCOCH_3$/$NHCOCH_3$), 1.25 (3 H, d, J = 6.6 Hz, γCH_3); HRCIMS m/z 204.0897, Δ 1.8 mmu for $C_8H_{14}NO_5$ (M + H)$^+$; LRCIMS (isobutane) m/z (% relative intensity) 204 (2.2), 172 (0.8), 144 (0.8), 79 (1.9), 69 (3.6).

***N*-Acetyl-*tert*-leucyl-3-methylisoleucine methyl ester (6).** *tert*-Leucine unit: ^1H NMR (CD_3OD) δ 4.35 (1 H, s, αH), 1.99 (3 H, s, $NHCOCH_3$), 0.99 (9 H, s, γCH_3); ^{13}C NMR (CD_3OD) δ 173.1 (2 C, s, $NHCOCH_3$/NHCO), 62.2 (d, αC), 35.2 (s, βC), 27.2 (3 C, s, γC), 22.4 (q, $NHCOCH_3$). 3-Methyl isoleucine unit: ^1H NMR (CD_3OD) δ 4.39 (1 H, s, αH), 3.67 (3 H, s, $COOCH_3$), 1.37 (2 H, m, γH), 0.95 (6 H, s, δCH_3/γCH_3), 0.86 (3 H, t, J = 7.5 Hz, γCH_3); ^{13}C NMR (CD_3OD) δ 173.1 (s, NHCO), 173.1 (s, $COOCH_3$), 60.6 (d, αC), 52.1 (q, $COOCH_3$), 37.5 (s, βC), 33.2 (t, γC), 24.0 and 23.9 (2 q, γ′C/γ″C), 8.4 (q, δC). *tert*-Leucine unit: ^1H NMR ($CDCl_3$) δ 6.09 (br d, NH), 4.27 (1 H, d, J = 9.2 Hz, αH), 2.00 (3 H, s, $NHCOCH_3$), 1.00 (9 H, s, γCH_3); ^{13}C NMR ($CDCl_3$) δ 171.5 (s, $NHCOCH_3$), 170.6 (s, CONH), 60.8 (d, αC), 34.8 (s, βC), 26.7 (3 C, s, γC), 23.3 (q, $NHCOCH_3$). 3-Methylisoleucine unit: ^1H NMR ($CDCl_3$) δ 6.09 (br d, NH), 4.46 (1 H, d, J = 9.1 Hz, αH), 1.31 (2 H, q, J = 7.5 Hz, γH), 0.92 and 0.91 (6 H, 2 s, γ′CH_3/γ″CH_3), 0.88 (3 H, t, δCH_3); ^{13}C NMR ($CDCl_3$) δ 169.8 (s, $COOCH_3$), 59.0 (d, αC), 51.8 (q, $COOCH_3$), 37.1 (s, βC), 32.1 (t, γC), 23.3 and 23.4 (2q, γ′C/γ″C), 8.1 (q, δC); HRCIMS (isobutane) m/z 315.2299, Δ 1.4 mmu for $C_{16}H_{31}N_2O_4$ (M + H)$^+$; LRCIMS (isobutane) m/z (% relative intensity) 315 (6.5), 283 (1.6), 244 (80.0), 160 (31.4), 128 (14.0), 100 (10.6), 86 (14.0).

Amino Acid Analysis. Polydiscamide A (450 μg) was heated in 6 N HCl (0.5 mL) for 16 h at 110 °C. The excess HCl was removed by passing a stream of N_2, and the residue was dried under vacuum. The dried residue was dissolved in the coupling buffer (CH_3CN–pyridine–Et_3N–H_2O, 10:5:2:3, 100 μL), phenyl isothiocyanate (5 μL) was added, and the reaction mixture kept at rt for 5 min. The excess reagents and the solvent were removed by the usual method. The residue was dissolved in H_2O/CH_3CN (7:2, 250 μL) and chromatographed by gradient reversed-phase C_{18} HPLC (5 μm, 4 mm × 25 cm) at 50 °C. The injections were made 5 min after the gradient program was started, and the peaks were detected at 254 nm. The following gradient program was used for solvent A [10 mM K_2HPO_4 (pH 6.5)] and B [10 mM K_2HPO_4 (pH 6.5)/CH_3CN (3:7)]: time (min)/flow (mL/min)/% A/% B: 0/1/100/0; 5/1/100/0; 15/1/91/9; 35/1/64/36; 38/1/0/100; 41/1/0/100; 44/1/100/0. The following amino acid peaks were detected. The retention time (min) and the ratio of area of the peak to the area of valine are given in parentheses. $Cys(SO_3H)$ (8.25, 1.10), Asn (9.04, 0.24), Thr (22.37, 0.73), Ala (22.79, 0.79), Pro (24.35, 1.92), Arg (24.90, 0.84), Val (30.53, 1.0), t-Leu (34.37, 0.49), Trp (38.31, 0.44).

1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) Derivatization and Absolute Stereochemistry. Polydiscamide A (1 mg) was treated with 6 N HCl (1 mL). The solution was twice frozen and degassed. The degassed mixture was heated at 110 °C for 18 h. The excess HCl was removed under vacuum, and a portion of the hydrolyzate was derivatized with FDAA. Standard amino acids (D and L) were derivatized separately. The derivatized hydrolyzate and standard amino acids were subjected to HPLC (reversed-phase C_{18}, 5μ, 4-mm × 15-cm column) using the following gradient program. Solvent A: 10% CH_3CN in 50 mM $(Et_3NH)_3PO_4$ at pH 3.0. Solvent B: 40% CH_3CN in 50 mM $(Et_3NH)_3PO_4$ at pH 3.0: time (min)/flow (mL/min)/% A/% B: 0/1/100/0; 5/1/100/0; 25/1/50/50; 50/1.5/0/100. The peaks were confirmed by co-injections and the use of different gradients. The following amino acids were confirmed. Retention times (min) are given in parentheses. D-Cysteic acid (16.83), L-Arg (19.61), L-Thr (21.14), D-Asp (24.05), L-Pro (26.40), D-Ala (29.47), D-Val (38.98), D-Trp (42.72), D-t-Leu (44.09), L-BrPhe (45.35).

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Supplementary Material Available: ^1H, ^1H–^1H COSY, COSYRCT, ^{13}C, DEPT, HETCOR, HMBC, NOESY, and 1D NOE NMR charts for 1, ^1H and ^{13}C NMR spectra for 2, and ^1H NMR spectra for 3–6 (46 pages). Ordering information is given in any current masthead page.

(^{13}C)-Substituted Erythronucleosides: Synthesis and Conformational Analysis by ^1H and ^{13}C NMR Spectroscopy

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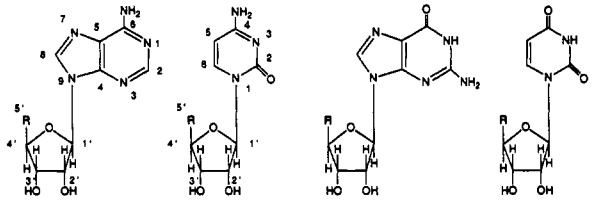
The erythronucleosides, 9-β-D-erythrofuranosyladenine (**1b**), 1-β-D-erythrofuranosylcytosine (**2b**), 9-β-D-erythrofuranosylguanine (**3b**), and 1-β-D-erythrofuranosyluracil (**4b**), were synthesized with and without ^{13}C-substitution at C1′ of the furanose ring. 75-MHz ^{13}C and 620-MHz ^1H NMR spectra of **1–4b** were interpreted, in the latter case with the assistance of spectral simulation, and ^1H–^1H, ^{13}C–^1H, and ^{13}C–^{13}C spin couplings were used to assess furanose conformation. $^3J_{HH}$ data in ^2H_2O were treated by computer to determine the preferred north and south conformers, their puckering amplitudes, and their mole fractions in solution, and J_{CH} data were used to complement this analysis. A similar treatment of spin coupling data for the corresponding ribonucleosides **1–4a** was also conducted to permit a comparison of furanose conformations in both series of compounds. Results show that the removal of the exocyclic hydroxymethyl group from **1–4a**, giving **1–4b**, significantly enhances the proportion of south conformers in aqueous (^2H_2O) solution.

Introduction

The ribonucleosides adenosine (**1a**), cytidine (**2a**), guanosine (**3a**), and uridine (**4a**) are the major constituent monomers of ribonucleic acid (RNA). Three interdependent[1] conformational domains exist in **1–4a** and have been



studied by a variety of experimental techniques.[2,3] These domains include the furanose ring, the exocyclic C5′ hydroxymethyl (CH_2OH) group, and the *N*-glycoside linkage. The conformational interdependence of these domains is illustrated, for example, by the coexistence of the *gg* hydroxymethyl conformation and anti *N*-glycoside bond conformation in certain ribonucleosides which apparently results from intramolecular hydrogen bonding. Thus, while the C5′ exocyclic hydroxymethyl group is a key determinant of furanose ring conformation in nucleosides, its presence is believed to have a global effect on the conformational behavior of these molecules in solution.

The erythronucleosides (9-β-D-erythrofuranosyladenine (**1b**), 1-β-D-erythrofuranosylcytosine (**2b**), 9-β-D-erythrofuranosylguanine (**3b**), and 1-β-D-erythrofuranosyluracil (**4b**)) are structural analogues of **1–4a**. Compounds **1–4b** contain the β-D-erythrofuranosyl ring in which the C5′ exocyclic CH_2OH group found in the β-D-ribofuranosyl ring of **1–4a** is replaced by a proton (H4′*R*). Hence, a comparison of the conformational properties of **1–4b** to those of **1–4a** may assist in evaluating the general effect of furanose ring substitution at C4′ on the solution conformations of nucleosides. Substitution effects can be important in determining the chemical and biological reactivities of nucleosides. For example, Van Roey et al.[4] have proposed recently that the anti-HIV activity of 3′-azido-2′,3′-dideoxythymidine (AZT) is related to the preferred south conformation of the furanose ring in this substituted nucleoside. This observation serves to emphasize the importance of understanding the structural factors that influence furanose ring conformation in biologically important molecules.

Four vicinal ^1H–^1H spin-coupling constants ($^3J_{HH}$) are available to assess the conformation of the β-D-erythrofuranosyl ring in **1–4b**: $^3J_{H1',H2'}$, $^3J_{H2',H3'}$, $^3J_{H3',H4'S}$, and $^3J_{H3',H4'R}$. Complementary structural information is supplied by ^{13}C–^1H and ^{13}C–^{13}C spin couplings, as described previously by Cyr and Perlin[5] and Serianni and Barker,[6] and these couplings are most easily and accurately measured in (^{13}C)-substituted compounds.[7] This report describes the chemical synthesis of natural and (1′-^{13}C)-substituted erythronucleosides **1–4b**, the interpretation of their ^1H and ^{13}C NMR spectra, and a conformational analysis of the furanose rings in **1–4b** based on ^1H–^1H, ^{13}C–^1H, and ^{13}C–^{13}C spin coupling constants. The preferred erythrofuranosyl ring conformations in **1–4b** are

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(1) Davies, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 135.
(2) For a comprehensive review, see: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(3) Van De Ven, F. J. M.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *178*, 1–38.
(4) Van Roey, P.; Salerno, J. M.; Chu, C. K.; Schinazi, R. F. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3929.
(5) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504.
(6) Serianni, A. S.; Barker, R. *J. Org. Chem.* **1984**, *49*, 3292.
(7) King-Morris, M. J.; Serianni, A. S. *J. Am. Chem. Soc.* **1987**, *109*, 3501.
(8) Kline, P. C.; Serianni, A. S. *J. Am. Chem. Soc.* **1990**, *112*, 7373.

0022-3263/92/1957-1772$03.00/0