couplings of a spin system. Consequently, spectra obtained with several values of t_d may have to be coadded to obtain the complete spectrum. Conversely, if several peaks do overlap, this dependency may be used to resolve them.

With this experiment, a high-resolution spectrum can be acquired using a magnetic field of such low homogeneity that a conventional spectrum would contain no useful information. It appears that this experiment is not bound by the limitations discussed above for the total spin coherence transfer echo technique in isotropic liquids. It is certainly superior to both zero-quantum coherence and SECSY spectra in that it produces single-quantum scalar coupling constants which would otherwise be unobtainable and simultaneously facilitates both the mapping of scalar coupling networks and the reassembly of the conventional (single-quantum coherence) spectrum of the spin system. We envisage that it will prove useful both in the context of in vivo NMR spectroscopy and in other areas for which low magnetic field homogeneity is unavoidable. Such applications, along with a detailed analysis of the pulse sequence will be given elsewhere.

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The Structure and Synthesis of Dolastatin 3[1a]

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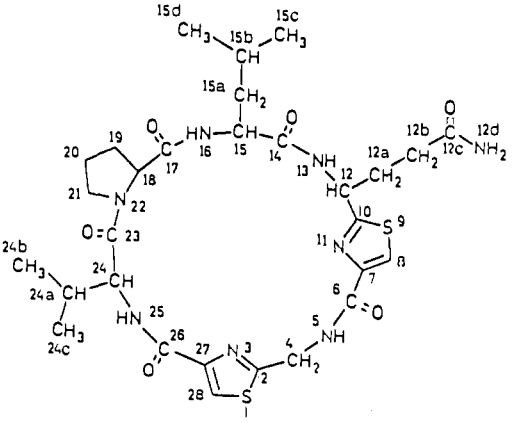
Because of the P388 lymphocytic leukemia (PS) cell growth inhibition displayed by dolastatin 3,[2] an unusual cyclic pentapeptide containing two thiazole amino acid units, its complete structure has been the focus of intensive interest. In 1979 we isolated ca. 1 mg of amorphous dolastatin 3 from 100 kg (wet wt) of the Indian Ocean sea hare *Dolabella auricularia*. On the basis of interpretation of results from the best instrumental techniques then available to us, and presuming an all L-configuration, structure **1** was tentatively assigned.[2] Later we[3a] and

cyclo[Pro-Leu-Val-(gln)Thz-(gly)Thz]
1

others[4] eliminated structure **1**, the chiral isomers,[3a,4] the reverse order of bonding,[3b,4] and a modified[3c,5] amino acid sequence by total syntheses.[6] When the remaining few micrograms of dolastatin 3 decomposed in storage, efforts directed at reisolation were begun and completed in mid 1986. Reisolation proved to be even more challenging and afforded only 1.8 mg [1.8×10^{-6}% yield, mp 155–159 °C, $[\alpha]^{29}_D$ −48.5 (*c* 0.01, CH_3OH)] of this sensitive peptide.[7] But by in-depth utilization of current advances in high field (400 and 500 MHz) ^1H NMR and other necessary techniques we now report unequivocal structure **2** for dolastatin 3 and total synthesis of this very interesting substance.

Acid hydrolysis (6 N HCl, 110 °C, 24–72 h) of natural (−)-dolastatin 3 (**2**) and examination of the hydrolysate (as *N*-trifluoroacetyl-*n*-butyl ester derivatives) by chiral gas chromatographic (fused silica column coated with "Chirasil-Val") analysis indicated that the Val, Pro, and Leu units all belonged to the L-configurational series. However, the configuration of (gln)Thz could not be ascertained and was solved by the synthetic routes to follow. Results of a two-dimensional NMR study[8] using ^1H,^1H



cyclo[L-Val-L-Pro-L-Leu-L-(gln)Thz-(gly)Thz], dolastatin 3
2

Table I. Dolastatin 3 Assignments in Deuteriochloroform Solution Relative to Tetramethylsilane for ^1H,^1H Connectivity by NOE Experiments

positn irradtd	^1H chemcl shift, ppm	connctvty by ^1H-[^1H] NOE diffrnce	% NOE
25	8.32	N25H, C24H	0.8
		N25H, C24cH	0.8
24	4.75	C24H, C24bH	1.1
		C24H, C24cH	1.1
		C24H, C21H	2.7
18	3.97	C18H, N16H	1.3
		C18H, C19H	0.8
16	6.01	N16H, C18H	4.1
		N16H, C15H	3.3
13	7.85	N13H, C15H	0.8
		N13H, N16H	0.8
		N13H, C12H	1.0
		N13H, C12aH	1.1
12	5.54	C12H, C12aH	2.3
		C12H, C12bH	1.3
12d	5.42	N12d, C12bH	0.5
	6.25	N12d, C12bH	0.7
5	8.76	N5H, C4H	1.2
		N5H, C4H	1.7
4	5.23	C4H, N5H	0.8

(1) (a) Antineoplastic agents series contribution 150: for part 149 consult: Daniel, L. W.; Parker, J.; Etkins, L. A.; Small, G. W.; Pettit, G. R. *J. Biol. Chem.*, submitted for publication. (b) Rand Afrikaans University, Johannesburg, Republic of South Africa. (c) Present address: University of Tennessee, Knoxville, TN 37996. (d) The Upjohn Co., Kalamazoo, MI 49001.

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(7) The physical properties (e.g., the 400-MHz ^1H NMR spectrum) were identical with those previously reported (ref 2).

0002-7863/87/1509-7581$01.50/0

COSY and ^{1}H-[^{1}H] NOE difference (Table I) experiments clearly allowed selection of sequence modification 2 for dolastatin 3. In turn, structure **2** corresponded to a sequence selected as a possibility based on the original high resolution mass spectral data.[2] Structure **2** was confirmed, and chirality of the (gln)Thz unit established by synthesis.

Synthesis of dolastatin 3 was conducted by one L-amino acid unit additions from L-Pro-OMe employing diethyl phosphorocyanidate[9] (DEPC)–triethylamine for peptide bond formation and *N*-Boc protection (trifluoroacetic acid cleavage). The thiazole amino acid components were prepared as previously summarized.[10] By this means, Boc-L-Leu-L-(gln)Thz-(gly)Thz-L-Val-L-Pro-OMe [mp 125–126 °C from ethyl acetate–hexane, $[\alpha]^{25.8}_D$ −74.9 (*c* 3.73, $CHCl_3$)] was obtained in 71% overall yield. After successive hydrolysis [1 N NaOH, dioxane 3 N HCl → mp 168–170 °C from ethanol–diethyl ether, $[\alpha]^{22}_D$ −37.7 (*c* 1.64, CH_3OH)], conversion (DCCI, DME, pentafluorophenol) to the OPfp active ester,[9] Boc cleavage, and cyclization[9,4c,11] (in dioxane containing 4% *tert*-butyl alcohol and 4-pyrrolidinopyridine at 95 °C, 76% yield), synthetic (−)-dolastatin 3 [colorless amorphous solid from ethanol–ethyl acetate, mp 170–173 °C, $[\alpha]^{25}_D$ −53.3 (*c* 0.94 in $CHCl_3$), ^{1}H and ^{13}C NMR entered in ref 12][13] was realized in 41% overall yield.

Analogous synthesis of an isomeric dolastatin 3 containing D-(gln)Thz in place of the L-epimer gave a product that was found to significantly differ from the natural product. The ^{1}H and ^{13}C NMR spectra of the D-(gln)Thz isomer suggested that considerable conformational changes resulted from this otherwise simple substitution.

The synthetic (−)-dolastatin 3 was identical with the natural product. Comparison ^{1}H NMR (400 MHz) spectra observed in methylene chloride-d_2 were superimposable as were SP-HRSIMS[14] spectra and thin-layer chromatographic comparisons (on silica gel, normal and reverse phase) in four different (e.g., 90:10:0.8 methylene chloride–methanol–water) solvent systems. Both specimens of dolastatin 3 inhibited growth of the PS leukemia[2] to the same extent (ED_{50} 0.16 vs 0.17 μg/mL)[15] and displayed an identical tendency to undergo decomposition in solution, especially in chloroform. The difficulties experienced in uncovering appropriate experimental conditions for cyclizing the linear pentapeptide precursor of (−)-dolastatin 3 combined with its sensitivity and biological activity suggests that its overall conformational preferences are very important. Other isomers of dolastatin 3 so far examined are quite stable and *in*active against the PS system.

The detailed structural elucidation and synthesis of dolastatin 3 reported herein now provides a pathway to further biological and chemical investigations of this important marine organism biosynthetic product.

Acknowledgment. The *summa summorum* of research support was provided by the Fannie E. Rippel Foundation, the Arizona Disease Control Research Commission, Herbert K. and Dianne Cummings (The Nathan Cummings Foundation, Inc.), Eleanor W. Libby, the Waddell Foundation (Donald Ware), and PHS Grant CA-16049-08-11 awarded by the National Cancer Institute, DHHS. For other assistance we are also pleased to thank Drs. C. Bass, H. Kizu, R. C. Kelly, and R. A. Nieman, P. L. Daschner, and NSF Grants CHE-8409644 and CHE-8620177.

Supplementary Material Available: ^{1}H NMR spectra (400 MHz) of natural and synthetic **2** as well as a 1:1 mixture of synthetic and natural **2** recorded in CD_2Cl_2 (3 pages). Ordering information is given on any current masthead page.

(8) A useful conformational analysis of the 350-MHz spectrum [in $(CD_3)_2SO$] of *cyclo*[Pro-Leu-Val-(gly)Thz-(gly)Thz] has been entered in ref 5.

(9) For leading references consult: Pettit, G. R. *Synthetic Peptides*; Elsevier: Amsterdam, The Netherlands, 1982, Vol. 6. Pettit, G. R. *Synthetic Peptides*; Elsevier: Amsterdam, The Netherlands, 1980; Vol. 5.

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(12) The following NMR results were obtained with a Bruker WP-500 instrument with use of a deuteriochloroform solution and tetramethylsilane as internal standard. Assignments were made based on extensive 2D NMR experiments such as HETCOR: ^{13}C NMR 18.55 (C-24c), 19.53 (C-24b), 21.18 (C-15d), 23.30 (C-15c), 25.43 (C-15b), 25.45 (C-15a), 28.41 (C-19), 29.62 (C-12a), 31.88 (C-24a), 33.27 (C-12b), 37.71 (C-20), 40.97 (C-4), 48.26 (C-21), 48.61 (C-12), 54.97 (C-15), 55.62 (C-24), 62.61 (C-18), 123.71 (C-28), 124.26 (C-8), 148.30 (C-2), 149.00 (C-10), 160.30 (C-27), 160.80 (C-7), 165.77 (C-12c), 169.4 (C-17), 170.92 (C-23), 171.09 (C-26), 172.00 (C-14), 174.30 (C-6); ^{1}H NMR (500 MHz) 0.90 (3 H, d, J = 6.6, H-15d), 0.95 (3 H, d, J = 6.6, H-15c), 1.04 (3 H, d, J = 6.7, H-24c), 1.15 (3 H, d, J = 6.8, H-24b), 1.53 (1 H, m, H-15b), 1.94 (2 H, m, H-20), 2.05 (1 H, m, H-24a), 2.13 (2 H, m, H-12a), 2.24 (2 H, m, H-19), 2.33 (2 H, m, H-15a), 2.53 (2 H, m, H-12b), 3.70 (1 H, m, H-21), 3.85 (2 H, m, H-21, H-15), 3.97 (1 H, dd, J = 7.9, H-18), 4.65 (1 H, dd, J = 18.0 and 2.0, H-4), 4.75 (1 H, dd, J = 9.0 and 7.0, H-24), 5.24 (1 H, dd, J = 18.0 and 7.2, H-4), 5.29 (1 H, br s, H-12d), 5.53 (1 H, ddd, J = 10.9, 9.1, 4.5, H-12), 6.02 (1 H, d, J = 6.6, H-16), 6.19 (1 H, br s, H-12d), 7.83 (1 H, d, J = 9.0, H-13), 8.05 (1 H, s, H-28), 8.07 (1 H, s, H-8), 8.30 (1 H, d, J = 9.4, H-25), and 8.73 (1 H, dd, J = 6.9 and 2.0, H-5) (J is measured in hertz).

(13) Satisfactory mass and NMR (^{1}H and ^{13}C) spectral and elemental analyses were obtained for each of the synthetic products. The following HR SP-SIMS results were obtained for dolastatin 3. Anal. Calcd for $C_{29}H_{41}N_8O_6S_2$ (M + H) 661.2591, found (natural) 661.2600 and found (synthetic) 661.2576.

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(15) The even stronger inhibition observed repeatedly with dolastatin 3 in 1979–1981 against the PS system might be due to a sensitivity difference in the present cell line and/or trace contamination of the original sample with an undetected and considerably more powerful dolastatin: see ref 2.