^{1}H AND ^{13}C NMR ASSIGNMENTS OF THE THIOPEPTIDE ANTIBIOTIC NOSIHEPTIDE

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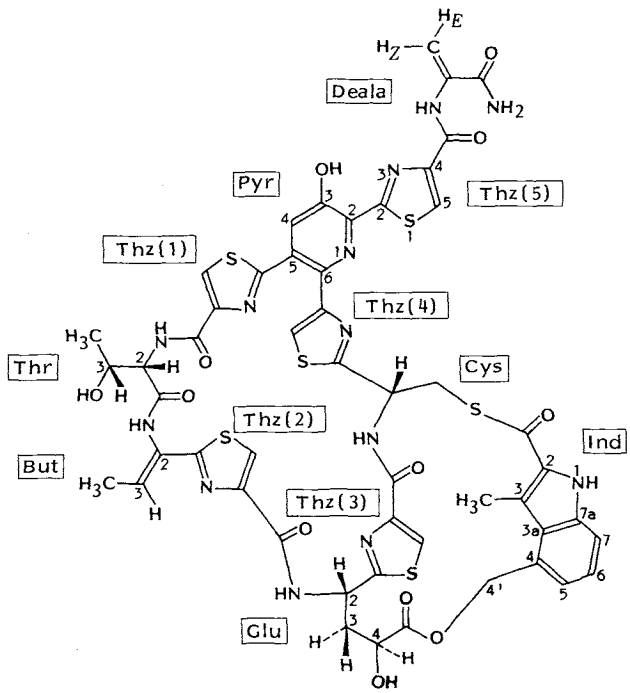
The ^{1}H and ^{13}C NMR spectra of nosiheptide have been assigned by use of 2D NMR techniques on unlabeled samples and biosynthetically multiple-labeled samples from stable isotope feeding experiments.

Nosiheptide (**1**, Fig. 1) is a sulfur-rich antibiotic which was isolated from *Streptomyces actuosus*[1,2]. It is very active *in vitro* against Gram-positive bacteria[3] and used widely as a feed additive for chicken and pigs to increase weight gains[4]. The complete structure of nosiheptide was elucidated by X-ray crystallography[1]. First NMR studies on **1** were reported by DEPAIRE *et al.*[5,6] in 1977 who partially assigned the ^{13}C NMR spectrum.

Extensive use of 2D NMR spectroscopy on both nonlabeled and biosynthetically multiple-labeled samples led to the complete and unambiguous ^{1}H and ^{13}C NMR assignments of nosiheptide, reported in this paper, as a prerequisite for our biosynthetic studies on this compound[7].

Fig. 1. Structure of nosiheptide (**1**).



Results and Discussion

^{1}H NMR Assignments

Several resonance assignments could be made from chemical shift and coupling constant considerations based on first-order inspection of the spectrum. The most upfield signal, a 6.43 Hz doublet at 0.95 ppm, could be assigned to the threonine methyl group and a singlet at 2.63 ppm to the methyl group of the indole moiety. The quartet at 6.46 ppm (J=6.95 Hz) and the doublet at 1.72 ppm (J=6.95 Hz) are assigned to the AX_3 spin system of dehydrobutyrine (But) 3-H and the But methyl group, respectively.

The indole (Ind) moiety shows the expected AMX-type spin system for three adjacent protons with signals at 7.12 ppm (d, J=7.02 Hz, Ind 5-H), 7.28 (dd, J=8.32 and 7.01 Hz, Ind 6-H) and 7.60 (d, J=8.35 Hz, Ind 7-H), although the expected long-range splitting between Ind 5-H and Ind 7-H was not resolved.

The use of 2D homonuclear correlation spectroscopy (COSY)[8,9] enabled us to assign most of the signals unambiguously, even in congested regions of the spectrum. For example, connectivity patterns determined from COSY shift correlations allowed the sequence of a set of signals starting at 4.09 (dd) and progressing through 1.80 (m)→2.44 (m)→5.63 (dd)→8.35 (d) to be assigned to Glu 4-H, two Glu 3-H, Glu 2-H and Glu NH, respectively. By working through sequential connectivity patterns of different spin systems other groups of signals were assigned to the protons of the cysteine and threonine moieties in a straightforward manner.

Signals for the methylene group of the dehydroalanine (Deala) residue are observed as two singlets at 5.76 and 6.37 ppm, both correlated in the COSY spectrum with the signal at 10.04 ppm (Deala NH). The calculation of chemical shifts according to the additivity rules[10] indicates that the signal of Deala 3-H_E is located more downfield than the signal of Deala 3-H_Z. Attempts to verify these assignments in nuclear Overhauser effect (NOE) experiments by irradiating Deala NH, Deala 3-H_Z and Deala 3-H_E were inconclusive. The results of the COSY experiment are summarized in Table 1.

Six sharp singlets in the aromatic region represent the protons of the five thiazole (Thz) rings and the pyridine (Pyr) proton. Four of these signals could be assigned by long range (delayed) COSY[11] correlations. The long range COSY contour plot at 320°K or higher temperatures shows correlations between protons which are 5, 6 and 7 bonds apart, *i.e.*, long range couplings between Thz(5) 5-H (8.55 ppm), Pyr 4-H (7.82) or Thz(4) 5-H (7.88) and Cys 2-H (5.88). The signal at 8.16 ppm which is correlated with the proton of But 3-H at 6.46 ppm was assigned to Thz(2) 5-H. Couplings between protons separated by such large numbers of bonds are favored by the conformational behavior and bond geometry of the molecule, and are not without precedent[12,13]. OH and NH protons like Thr OH, Thr NH, Cys NH and Glu NH were identified after exchange with D_2O at 320 and 350°K. Most exchangeable protons showed also COSY correlations. Pyr OH and Deala NH_2 could not be assigned because their signals were not observed in these spectra.

In this way, all proton signals except the two singlets at 8.30 and 8.65 ppm (see below) could be assigned unequivocally (Table 1).

^{13}C NMR Assignments

DEPAIRE *et al.*[5] observed signals for 29 quaternary (sp^2), 10 methine (sp^2), 1 methylene (sp^2), 5 methine (sp^3), 3 methylene (sp^3) and 3 methyl carbon atoms in the single frequency off resonance decoupled ^{13}C NMR spectrum of **1**. An edited distortionless enhancement by polarization transfer (DEPT)[14] analysis

Table 1. 2D NMR COSY shift correlations of nosiheptide.

0.95[a]	Thr CH_3↔ 4.00[a]	Thr 3-H	5.76	Deala 3-H_Z↔ 6.37	Deala 3-H_E
1.72	But CH_3↔ 6.46	But 3-H		↔10.04	Deala NH
	↔ 9.32	But NH	5.88	Cys 2-H↔ 3.56	Cys 3-H
1.80	Glu 3-H↔ 4.09	Glu 4-H		↔ 3.86	Cys 3-H
	↔ 5.63	Glu 2-H		↔ 7.72	Cys NH
2.44	Glu 3-H↔ 4.09	Glu 4-H	6.37	Deala 3-H_E↔ 5.76	Deala 3-H_Z
	↔ 5.63	Glu 2-H		↔10.04	Deala NH
3.56	Cys 3-H↔ 3.86	Cys 3-H	6.46	But 3-H↔ 1.72	But CH_3
	↔ 5.88	Cys 2-H		↔ 9.32	But NH
3.86	Cys 3-H↔ 3.56	Cys 3-H	7.12	Ind 5-H↔ 5.59	Ind 4′-H
	↔ 5.88	Cys 2-H		↔ 7.28	Ind 6-H
4.00	Thr 3-H↔ 0.95	Thr CH_3		↔ 7.60	Ind 7-H
	↔ 4.57	Thr 2-H	7.28	Ind 6-H↔ 7.12	Ind 5-H
4.09	Glu 4-H↔ 1.80	Glu 3-H		↔ 7.60	Ind 7-H
	↔ 2.44	Glu 3-H	7.58	Thr OH↔ 4.57	Thr 2-H
4.57	Thr 2-H↔ 4.00	Thr 3-H	7.60	Ind 7-H↔ 7.12	Ind 5-H
	↔ 7.58	Thr OH		↔ 7.28	Ind 6-H
	↔ 7.64	Thr NH	7.64	Thr NH↔ 4.57	Thr 2-H
5.40	Ind 4′-H↔ 5.59	Ind 4′-H	7.72	Cys NH↔ 5.88	Cys 2-H
5.59	Ind 4′-H↔ 5.40	Ind 4′-H	8.35	Glu NH↔ 5.63	Glu 2-H
	↔ 7.12	Ind 5-H	9.32	But NH↔ 1.72	But CH_3
5.63	Glu 2-H↔ 1.80	Glu 3-H		↔ 6.46	But 3-H
	↔ 2.44	Glu 3-H	10.04	Deala NH↔ 5.76	Deala 3-H_Z
	↔ 8.35	Glu NH		↔ 6.37	Deala 3-H_E

[a] ppm.

Table 2. ^1H-^{13}C COSY correlations of nosiheptide at 320°K.

Assignment	^{13}C chemical shift (ppm)	^1H chemical shift (ppm)	Assignment	^{13}C chemical shift (ppm)	^1H chemical shift (ppm)
Ind CH_3	12.23	2.63	Thr 3	66.50	4.00
Ind 5	123.23	7.12	Cys 2	49.05	5.88
Ind 6	124.91	7.28	Cys 3	29.49	3.56, 3.86
Ind 7	114.40	7.60	Glu 2	45.15	5.63
Ind 4′	65.90	5.40, 5.59	Glu 3	37.60	1.80, 2.44
Deala 3	103.60	5.76, 6.37	Glu 4	66.40	4.09
But CH_3	13.50	1.72	Pyr 4	127.12	7.82
But 3	128.85	6.46	Thz(2) 5	124.45	8.16
Thr CH_3	18.25	0.95	Thz(4) 5	119.98	7.88
Thr 2	56.57	4.57	Thz(5) 5	126.80	8.55

confirmed this result, demonstrating carbon types and numbers to be also consistent with the X-ray analysis[1].

Correlations of the ^1H and ^{13}C chemical shifts for 20 of the 22 protonated carbons by their one bond couplings were established through a 2D ^1H-^{13}C-COSY[15] experiment as shown in Table 2.

Assignment of most of the quaternary carbons could be achieved by a series of 2D-COLOC[16] experiments optimized for different long-range coupling constants (data summarized in Table 3) and by comparison of the ^{13}C NMR data for **1** with literature values for model compounds which had been obtained by acid and alkaline hydrolysis of nosiheptide[17]. By comparison with the ^{13}C assignments of such a model compound[17], resonance signals of the indole moiety could be assigned to Ind C-3 (118.35 ppm), Ind C-3a (124.70), Ind C-4 (129.20), Ind C-2 (130.40) and Ind C-7a (137.60). The assignments of Ind C-3,

Table 3. COLOC correlations of ^{13}C-^{1}H long range couplings of nosiheptide.

^{1}H assignment (ppm)	^{13}C assignment (ppm)		
	4 Hz	6 Hz	9 Hz[a]
Ind CH_3 (2.63)		Ind C-3 (118.35)	Ind C-3 (118.35)
	Ind C-2 (130.40)	Ind C-2 (130.40)	Ind C-2 (130.40)
Deala 3-H_Z (5.76)	Deala C-2 (134.26)	Deala C-2 (134.26)	Deala C-2 (134.26)
	Deala CO (165.00)	Deala CO (165.00)	Deala CO (165.00)
Pyr 4-H (7.82)	Pyr C-4 (127.12)		Pyr C-4 (127.12)
	Pyr C-2 (135.00)	Pyr C-2 (135.00)	Pyr C-2 (135.00)
		Pyr C-6 (142.52)	Pyr C-6 (142.52)
		Thz(1) C-2 (163.85)	Thz(1) C-2 (163.85)
Ind 6-H (7.28)		Ind C-7a (137.60)	
Thz(4) 5-H (7.88)	Thz(4) C-4 (153.10)	Thz(4) C-4 (153.10)	Thz(4) C-4 (153.10)
		Thz(4) C-2 (168.98)	Thz(4) C-2 (168.98)
Thz(2) 5-H (8.16)	Thz(2) C-4 (147.62)	Thz(2) C-4 (147.62)	Thz(2) C-4 (147.62)
		Thz(2) C-2 (166.32)	Thz(2) C-2 (166.32)
Thz(3) 5-H (8.30)	Thz(3) C-4 (148.70)	Thz(3) C-4 (148.70)	Thz(3) C-4 (148.70)
		Thz(3) C-2 (170.04)	Thz(3) C-2 (170.04)
Thz(5) 5-H (8.55)	Thz(5) C-4 (149.57)	Thz(5) C-4 (149.57)	Thz(5) C-4 (149.57)
		Thz(5) C-2 (167.10)	Thz(5) C-2 (167.10)
Thz(1) 5-H (8.65)	Thz(1) C-4 (149.83)	Thz(1) C-2 (163.85)	

[a] The coupling constant values for the 3 COLOC experiments given represent the average long-range C-H coupling constant to which the experiment was tuned and should be interpreted to indicate true values of the coupling constants. The range of the J_{CH} found in the COLOC experiments are consistent with actual values measured in the gated ^{13}C NMR spectrum.

Ind C-2 and Ind C-7a are confirmed by two-bond and three-bond coupling correlations. The Deala C-2 and Deala CO resonances can also be differentiated by long range correlations (COLOC): Deala C-2 resonates at 134.26 ppm while Deala CO appears at 165.00.

Among the four quaternary carbon atoms in the pyridine moiety, signals at 129.90, 135.00 and 142.52 ppm can be assigned to Pyr C-5, Pyr C-2 and Pyr C-6, respectively, based on the ^{1}H-^{13}C long-range coupling patterns (3 bond coupling) and the ^{13}C NMR data of a model compound[17].

The COLOC correlations within each thiazole ring indicate that the Thz 5-H displays a two-bond coupling with Thz C-4 and a three-bond coupling with Thz C-2. As a consequence, the five signals at 153.10 (Thz(4)), 149.83 (Thz(1)), 149.57 (Thz(5)), 148.70 (Thz(3)) and 147.62 ppm (Thz(2)) are assigned to the carbons of the Thz C-4 groups as well as another five signals at 170.04 (Thz(3)), 168.98 (Thz(4)), 167.10 (Thz(5)), 166.32 (Thz(2)) and 163.85 ppm (Thz(1)) to the Thz C-2 carbons. The ^{13}C resonance at 163.85 ppm was assigned to Thz(1) C-2, based on its long-range coupling with Pyr 4-H at 7.82 ppm. An additional long-range correlation of Thz(1) C-2 at 163.85 ppm with the proton signal at δ 8.65 allowed the latter signal to be assigned to Thz(1) 5-H. Therefore, the only remaining signal in the proton spectrum can be assigned to Thz(3) 5-H (8.30 ppm).

The four carbonyl carbons adjacent to the thiazole rings could not be differentiated by the aforementioned methods, and we turned to biosynthetically ^{13}C-labeled samples to help assign these additional signals. Our biosynthetic results had shown that the thiazole rings derive from L-cysteine and the carboxyl group of another amino acid[7]. We therefore fed L-[1,2-$^{13}C_2$]serine, a precursor of cysteine. The sample of **1** from this experiment showed the expected enrichment and coupling of the Thz C-4 carbons (from carbon 2 of serine) and the adjacent carbonyl carbons (from carbon 1 of serine). A 2D-INADEQUATE[18~20] experiment gave correlations between the previously assigned Thz C-4 carbons

and the carbonyl carbons, which allowed an easy assignment of the latter.

Thz(1) C-4 (149.83 ppm)↔(159.45 ppm) Thz(1) CO

Thz(2) C-4 (147.62 ppm)↔(159.60 ppm) Thz(2) CO

Thz(3) C-4 (148.70 ppm)↔(159.80 ppm) Thz(3) CO

Thz(5) C-4 (149.57 ppm)↔(158.20 ppm) Thz(5) CO

Additional correlations were observed which confirmed assignments already established.

Another feeding experiment with L-[2,3-$^{13}C_2$]serine took advantage of the fact that serine is also incorporated into the pyridine[7], threonine and glutamic acid moieties†. The correlations in the 2D-INADEQUATE spectrum allowed the assignments of Pyr C-3 (from carbon 3 of serine), Thr CO and Glu CO (both from carbon 2 of serine).

Pyr C-2 (135.00 ppm)↔(150.80 ppm) Pyr C-3

Thr CO (167.69 ppm)↔(56.57 ppm) Thr C-2

Glu CO (172.62 ppm)↔(66.40 ppm) Glu C-4

Again, additional correlations were observed which confirmed assignments already made based on other data discussed above.

The two remaining signals in the ^{13}C NMR spectrum can be assigned based on their chemical shifts to But C-2 (129.27 ppm) and Ind CO (181.80 ppm).

The complete ^{1}H and ^{13}C NMR assignments for nosiheptide are summarized in Table 4.

Table 4. ^{1}H and ^{13}C NMR spectral data of nosiheptide (δ in ppm, J in Hz).

Ind CO	181.80 s	But 3	128.85 d	6.46 (q, J=6.95)
Glu CO	172.62 s	Pyr 4	127.12 d	7.82 (s)
Thz(3) 2	170.04 s	Thz(5) 5	126.80 d	8.55 (s)
Thz(4) 2	168.98 s	Thz(1) 5	125.98 d	8.65 (s)
Thr CO	167.69 s	Thz(3) 5	125.25 d	8.30 (s)
Thz(5) 2	167.10 s	Ind 6	124.91 d	7.28 (dd, J=7.01, 8.32)
Thz(2) 2	166.32 s	Ind 3a	124.70 s	
Deala CO	165.00 s	Thz(2) 5	124.45 d	8.16 (s)
Thz(1) 2	163.85 s	Ind 5	123.23 d	7.12 (d, J=7.02)
Thz(3) CO	159.80 s	Thz(4) 5	119.98 d	7.88 (s)
Thz(2) CO	159.60 s	Ind 3	118.35 s	
Thz(1) CO	159.45 s	Ind 7	114.40 d	7.60 (d, J=8.35)
Thz(5) CO	158.20 s	Deala 3	103.60 t	6.37 *E* (s), 5.76 *Z* (s)
Thz(4) 4	153.10 s	Thr 3	66.50 d	4.00 (m)
Pyr 3	150.80 s	Glu 4	66.40 d	4.09 (dd, J=2.24, 11.89)
Thz(1) 4	149.83 s	Ind 4′	65.90 t	5.59 (d, J=11.75), 5.40 (d, J=11.71)
Thz(5) 4	149.57 s	Thz 2	56.57 d	4.57 (dd, J=3.75, 7.55)
Thz(3) 4	148.70 s	Cys 2	49.05 t	5.88 (ddd, J=4.89, 5.21, 9.67)
Thz(2) 4	147.62 s	Glu 2	45.15 d	5.63 (dd, J=2.37, 11.86)
Pyr 6	142.52 s	Glu 3	37.60 t	2.44 *S* (m), 1.80 *R* (m)
Ind 7a	137.60 s	Cys 3	29.49 t	3.86 (dd, J=4.89, 13.86), 3.56 (dd, J=5.49, 13.95)
Pyr 2	135.00 s	Thr CH_3	18.25 q	0.95 (d, J=6.43)
Deala 2	134.26 s	But CH_3	13.50 q	1.72 (d, J=6.95)
Ind 2	130.40 s	Ind CH_3	12.23 q	2.63 (s)
Pyr 5	129.90 s			
But 2	129.27 s			
Ind 4	129.20 s			

Ind NH 11.19 (s, br); Deala NH 10.04 (s); But NH 9.32 (s); Glu NH 8.35 (d, J=8.42); Glu OH 8.00 (s, br); Cys NH 7.72 (d, J=9.67); Thr NH 7.64 (d, J=8.01); Thr OH 7.58 (s, br).

† A detailed discussion of these results will be the subject of a future publication.

Experimental

^{1}H and ^{13}C NMR spectra were acquired in DMSO-d_6 at a field strength of 7.1 T on Bruker WM-300 or IBM AF-300 spectrometers. Samples were prepared in 5 mm tubes, and were heated to 320°K in the probe prior to analysis. Spectra were internally referenced to the solvent resonance.

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