A37812: *N*-METHYLSTREPTOTHRICIN F

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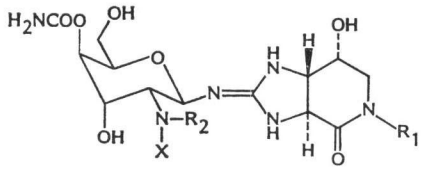
A37812, a new member of the streptothricin class of antibiotics, has been isolated and characterized as *N*-methylstreptothricin F. The structure elucidation of A37812 is based on results from ^{13}C and ^{1}H NMR spectroscopies.

The streptothricin antibiotics are a group of potent but usually rather toxic molecules having the general structure **1**.



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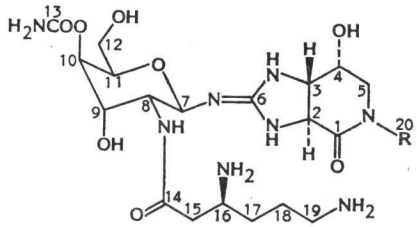


A37812 (**2**) R=CH_3
Streptothricin F (**3**) R=H

Various streptothricins differ by having either R_1 or R_2=CH_3 in some cases and H in other cases, or by having differences in the group X. The most thoroughly studied structure is that of streptothricin F, for which R_1=R_2=H and X=β-lysine.[1] We have isolated and characterized a new member of the streptothricin family, antibiotic A37812; A37812 has been examined by fast atom bombardment mass spectrometry and by ^{13}C and ^{1}H NMR spectroscopy. The spectroscopic data indicate that A37812 has the structure **2**.

Results and Discussion

Streptothricin F (**3**) is a well-characterized antibiotic; most of its structural features have been known for many years,[1,2] and the last structural detail — the location of the carbamate group on the carbon-10 hydroxyl — has recently been defined.[3,4] Both ^{13}C NMR[5,6,7] and ^{1}H NMR[8] studies have been described for antibiotics of the streptothricin family.

The ^{1}H NMR spectrum of A37812, shown in Fig. 1, contains all the resonances appropriate for streptothricin F, plus the peak 20 — a triply-intense singlet with a chemical shift suggestive of a methyl group on nitrogen. The fast atom bombardment mass spectral data confirm that A37812 ($C_{20}H_{36}N_8O_8$) differs in mass from streptothricin F ($C_{19}H_{34}N_8O_8$) by the equivalent of a methyl group. The ^{1}H NMR parameters illustrated in Fig. 1 are listed in Table 1, where they are compared with results for the gulosamine protons of streptothricin F[3] and the streptolidine resonances of a related streptothricin, LL-AC541 (**1**, R_1=H, R_2=CH_3, X=$\overset{O}{\overset{\|}{C}}CH_2NHCH{=}NH$).[8] The close similarity of chemical shifts

Fig. 1. 360 MHz ^1H NMR spectrum of A37812 in D_2O.

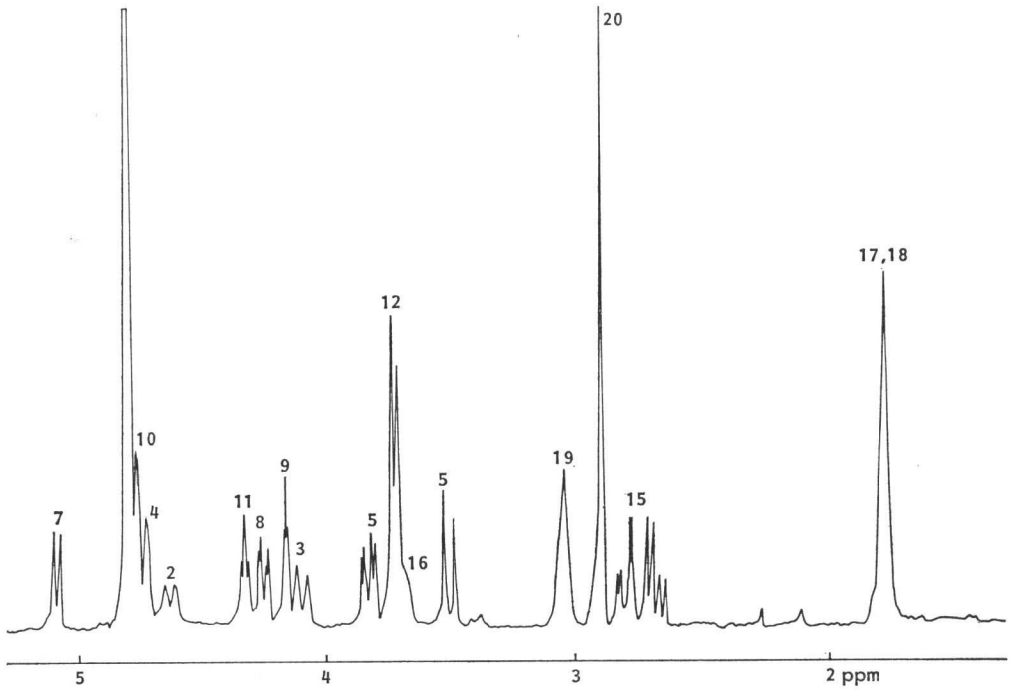


Table 1. ^1H NMR parameters for A37812 and other streptothricins in D_2O.

Position	Chemical shifts, ppm (*J*, Hz)		
	A37812[a]	Streptothricin F[b]	LL-AC541[c]
2	4.62 (14.5, small)		4.69 (14.8)
3	4.09 (14.5, small)		4.12 (14.8, 2.7)
4	4.71 (5.5, others)		4.73 (5.5, 2.7)
5	3.82 (14.5, 5.5)		3.85 (15.0, 5.5)
5	3.50 (14.5, small)		3.42 (15.0, 1.2)
7	5.08 (10)	5.14 (9.7)	5.36
8	4.24 (10, 3)	4.28 (9.7, 3.4)	
9	4.15 (3, 4)	4.20 (3.4, 3.4)	
10	4.75 (4, small)	4.80 (3.4)	
11	4.32 (6, small)	4.36 (6.0)	
12	3.71 (6)	3.75 (6.0)	3.78
15	2.80 (17, 4)		
15	2.67 (17, 8)		
16	3.69		
17	1.78		
18	1.78		
19	3.04		
20	2.89		

[a] A37812 spectrum in D_2O.

[b] Ref 3: streptothricin F hydrochloride in D_2O.

[c] Ref 8: LL-AC541 in D_2O, chemical shifts adjusted by subtraction of 0.47 ppm from values given in Tables 3 and 4 of ref 8. LL-AC541=**1** with R_1=H, R_2=CH_3, and X=$\overset{O}{\overset{\|}{C}}CH_2NHCH{=}NH$.

Fig. 2. 62.9 MHz ^{13}C NMR spectrum of A37812 in 2% pyridine-d_5 in D_2O.

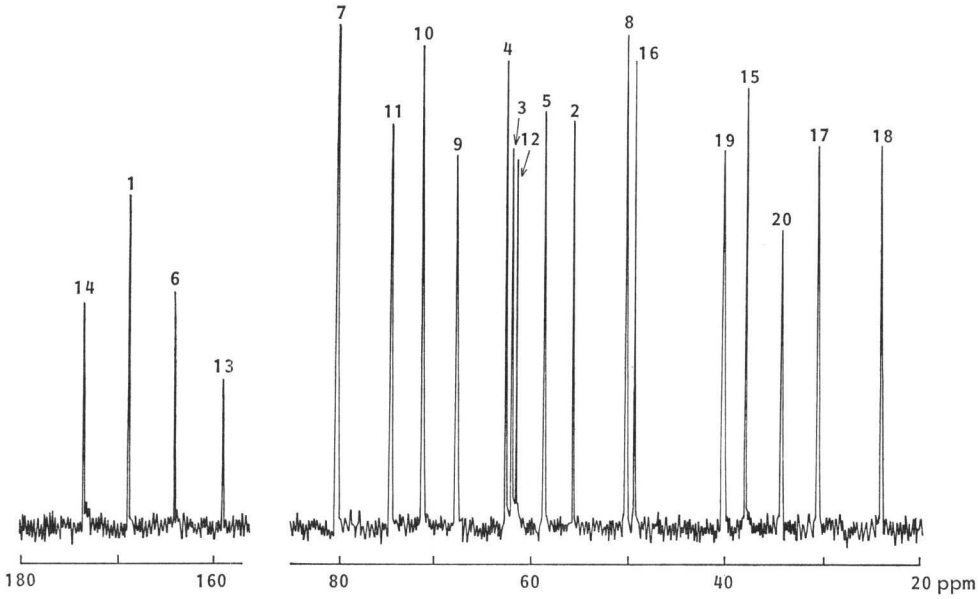


Table 2. Comparison of ^{13}C NMR chemical shifts for A37812 and streptothricin F.

Assignment	Racemomycin A[a]	Streptothricin F[b]	A37812[c]	$\Delta\delta$[d]
14	172.8	173.1	173.2 s	+0.1
1	170.7	171.1	168.7 s	−2.4
6	163.5	163.7	163.8 s	+0.1
13	158.8	158.7	158.7 s	0.0
7	79.8	79.9	80.0 d	+0.1
11	74.3	74.5	74.4 d	−0.1
10	70.8	71.0	71.1 d	+0.1
9	67.2	67.4	67.5 d	+0.1
3	62.1	62.3	61.7 d	−0.6
4	61.6	61.9	62.3 d	+0.4
12	61.1	61.3	61.2 t	−0.1
2	55.1	55.5	55.6 d	+0.1
5	50.1	50.2	58.4 t	+8.6
8	49.8	50.0	50.0 d	0.0
16	49.1	49.3	49.2 d	−0.1
19	39.8	40.0	40.0 t	0.0
15	37.1	37.6	37.7 t	+0.1
17	29.8	30.3	30.3 t	0.0
18	23.7	23.9	23.9 t	0.0
20	—	—	34.0 q	—

[a] Spectrum in D_2O with internal dioxane; δ (dioxane)=67.4 ppm. Ref 6.

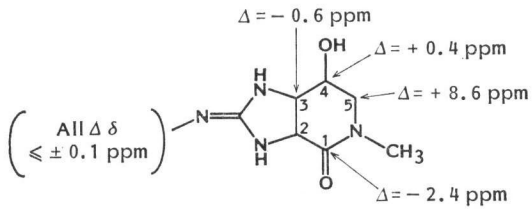
[b] Spectrum in D_2O containing 2% pyridine; middle pyridine signal initially taken as 135.5 ppm. However, the values listed above have been adjusted by adding 3.4 ppm in order to bring them into closest agreement with those for A37812. Ref 5.

[c] Spectrum in D_2O containing 2% pyridine-d_5. Chemical shifts calculated with reference to dioxane in D_2O plus 2% pyridine-d_5; δ (dioxane)=67.4 ppm. The middle pyridine triplet in the A37812 spectrum has a chemical shift of 138.4 ppm by this procedure. The multiplicities were observed in a gated decoupled spectrum; carbon resonances between 37 ppm and 80 ppm were assigned on the basis of single-frequency decoupling experiments, using the proton assignments in Table 1.

[d] $\Delta\delta=\delta$(A37812)$-\delta$(streptothricin F).

and coupling constants for the compounds listed in Table 1 indicates that in A37812 the carbons of the streptolidine and gulosamine moieties have the same relative configurations as do the corresponding sites in the other two streptothricins.

The ^{13}C NMR spectrum of A37812 in 2% pyridine-d_5 in D_2O is shown in Fig. 2; the spectrum contains 20 resonances. The "extra" peak (in addition to the 19 resonances which are similar to those in the ^{13}C spectrum of streptothricin F) is a quartet at 34.0 ppm — confirming the suggestion of a methyl group on nitrogen. The A37812 carbon resonances have been assigned unambiguously by selectively decoupling most of the resonances in the A37812 proton spectrum; these assignments are shown in Fig. 2 and also in Table 2, where they are compared with ^{13}C chemical shift values for streptothricin F[5] (=racemomycin A[6]). The chemical shift changes listed in the last column of Table 2,



$\Delta\delta$, indicate that A37812 has the structure **2**, with the new CH_3 group on the lactam nitrogen of the streptolidine moiety. The carbons of the gulosamine and β-lysine portions of structures **1** and **2** have chemical shifts which are virtually identical; only carbons 1, 3, 4, and 5 show chemical shift changes of more than $\pm$0.1 ppm on going from streptothricin F to A37812.

Table 3. *In vitro* antimicrobial spectrum of A37812.

Organism	MIC (μg/ml)
Staphylococcus aureus X400	8
S. aureus S13E	8
Streptococcus pneumoniae PARK I	8
S. pyogenes C203	8
Escherichia coli N10	8
E. coli EC14	16
E. coli TEM	4
Klebsiella pneumoniae X26	2
K. pneumoniae KAE	4
K. pneumoniae X68	4
Enterobacter aerogenes C32	4
E. cloacae EB5	8
Salmonella sp. X514	8
Pseudomonas aeruginosa X239	>128
Serratia marcescens X99	32
Shigella sonnei N9	16
Proteus inconstans PR33	>128
P. morganii PR15	4

Antibiotic A37812 exhibits antimicrobial activity typical of other members of the streptothricin family,[9,10,11] including good activity against strains of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and other organisms listed in Table 3.

Experimental

Culture A37812, an actinomycete, was grown in 100 liters of fermentation medium containing glycerol 2.5%, Nutrisoy grits 1.5%, blackstrap molasses 0.3%, casein 0.1% and $CaCO_3$ 0.25%; the culture was fermented at 25°C for 3 days.

Isolation of A37812

Fermentation broth (200 liters) of culture A37812 was filtered through a filter press, and the filtrate was passed over a column containing 10 liters of activated carbon (Pittsburgh Activated Carbon Company, 12×40). The column was washed with water, and the active material was eluted with acetone - 0.005 N HCl (35: 65); the elution was monitored using *Bacillus subtilis* bioassay. The active fractions were combined, concentrated to remove the acetone, and lyophilized to yield crude A37812 containing a major active component and several minor active components. The crude A37812 (106 g) was dissolved in 600 ml water and passed over a 5.7×60 cm column of Duolite ES-762 resin packed in water. The column was washed with water, and the elution of activity was followed by bioassay.

The highest-potency fractions were combined and lyophilized to yield 60 g of semi-purified A37812. The semi-purified A37812 was recycled over another Duolite ES-762 column to further increase the purity.

The partially purified A37812 was a hygroscopic yellowish powder; this material (5.77 g) was dissolved in water and injected onto a 2.5×60 cm stainless steel column containing LP-1 silica equilibrated with methanol - 1.5 N NH_4OH (4: 1). After collecting two load volumes of effluent, the solvent was changed to methanol - water (4: 1) and the column was washed until the conductivity and UV absorption had returned to baseline values; the isolation was monitored at λ 225 nm (2.0 aufs). The active material was then eluted with methanol - 0.5 N HCl (4: 1). Essentially 100% of the activity was contained in 2.26 g of purified antibiotic recovered in three fractions.

A second chromatographic step used a pre-packed silica column (E. Merck, size B), equilibrated and washed as before with methanol - NH_4OH and methanol - water. This system was loaded with 613 mg of purified antibiotic and the active material was eluted with methanol - 0.2 N HCl (4: 1), yielding an active center cut of 295 mg. This material was rechromatographed on a Merck size A column (using the second set of solvents); 65 mg of a white powder was recovered after lyophilization of two center-cut fractions. The A37812 produced from this procedure was examined by spectroscopic methods and characterized as *N*-methylstreptothricin F.

Mass Spectrometry

The FAB mass spectrum of A37812 gives an $(M+H)^+$ ion at m/z=517.2733; ($C_{20}H_{36}N_8O_8$+H)= 517.2734 (theoretical).

^{1}H NMR Spectroscopy

^{1}H NMR spectra of A37812 were recorded in D_2O solution at 360 MHz; chemical shifts are reported vs. external TSP (capillary). The spectrum was fully decoupled.

^{13}C NMR Spectroscopy

^{13}C NMR spectra of A37812 were recorded in D_2O solution containing 2% pyridine-d_5; spectrometer frequency=62.9 MHz. The spectrum was assigned unambiguously by selectively decoupling most of the resonances in the proton spectrum of A37812; other experimental details are given in footnote c of Table 2.

References

1) Van Tamelen, E. E.; J. R. Dyer, H. A. Whaley, H. E. Carter & G. B. Whitfield, Jr.: Constitution of streptolin-streptothricin group *Streptomyces* antibiotics. J. Am. Chem. Soc. 83: 4295~4296, 1961
2) Bycroft, B. W. & T. L. King: Crystal structure of streptolidine, a guanidine-containing amino acid. J. Chem. Soc. Chem. Commun. 1972: 652~653, 1972
3) Kusumoto, S.; Y. Kambayashi, S. Imaoka, K. Shima & T. Shiba: Total chemical structure of streptothricin. J. Antibiotics 35: 925~927, 1982
4) Kusumoto, S.; S. Imaoka, Y. Kambayashi & T. Shiba: Total synthesis of antibiotic streptothricin F. Tetrahedron Lett. 23: 2961~2964, 1982
5) Gould, S. J.; K. J. Martinkus & C.-H. Tann: Biosynthesis of streptothricin F. 1. Observing the interaction of primary and secondary metabolism with [1,2-$^{13}C_2$] acetate. J. Am. Chem. Soc. 103: 2871~2872, 1981
6) Sawada, Y.; S. Kawakami, H. Taniyama & Y. Inamori: Incorporation of carboxyl and methyl carbon-13 labeled acetates into racemomycin A by *Streptomyces lavendulae* ISP 5069. J. Antibiotics 30: 630~632, 1977
7) Kawamura, T.; T. Kimura, K. Tago, T. Beppu & K. Arima: The identity of S15-1-A and B with racemomycins A and C. J. Antibiotics 29: 844~846, 1976
8) Borders, D. B.; K. J. Sax, J. E. Lancaster, W. K. Hausmann, L. A. Mitscher, E. R. Wetzel & E. L. Patterson: Structures of LL-AC541 and LL-AB664, new streptothricin-type antibiotics. Tetrahedron 26: 3123~3133, 1970
9) Lumb, M.; N. Chamberlain, T. Cross, P. E. Macey, J. Spyvee, J. M. Uprichard & R. D. Wright: An antibiotic complex (A4788) containing streptothricin and having activity against powdery mildews. J. Sci. Food Agric. 13: 343~352, 1962

10) TSURUOKA, T.; T. SHOUMURA, N. EZAKI, T. NIWA & T. NIIDA: SF-701, a new streptothricin-like antibiotic. J. Antibiotics 21: 237~238, 1968

11) SAWADA, Y.; S. KAWAKAMI, H. TANIYAMA, K. HAMANO, R. ENOKITA, S. IWADO & M. ARAI: Glycinothricin, a new streptothricin-class antibiotic from *Streptomyces griseus*. J. Antibiotics 30: 460~467, 1977