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Complete ^{1}H and ^{13}C NMR Assignments of the Oligosaccharide Antibiotic Sch 27899

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The complete assignments of ^{1}H and ^{13}C data for Sch 27899 are described. The compound is an oligosaccharide antibiotic belonging to the class everninomicin. It has a molecular mass of 1629. The assignments are based on 2D HMQC, HMQC-TOCSY and HMBC experiments. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Sch 27899, an antibiotic produced[1] by *Micromonospora carbonacea*, is highly active against Gram-positive bacteria including those which are resistant to methicillin and vancomycin. Although the exact mechanism of action is not full understood, it is being investigated in the clinic as a drug candidate against resistant bacteria. Sch 27899 belongs to the class of oligosaccharide antibiotics everninomicins,[2,3] characterized by the presence of two *ortho* ester carbon atoms, aromatic esters and several sugar residues. The structure of Sch 27899 (Fig. 1) was established by chemical degradation and spectroscopic evidence.[4] In this paper, we report the complete proton and carbon assignments, which are essential for the structural elucidation of minor components in the fermentation broth, degradation products and products of chemical modifications. The structures of these related compounds are important for the clinical development of Sch 27899 as a drug candidate.

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EXPERIMENTAL

Sample preparation

The central *ortho* ester at C-16 is very acid labile. In order to avoid decomposition, the $CDCl_3$ solvent was passed through a small column of basic alumina to remove any residual acid. Sch 27899 is not very soluble in $CDCl_3$, so a mixed solvent of $CDCl_3$–CD_3OD is used. The chemical shifts of the methine carbons attached to hydroxyl groups are dependent on the CD_3OD content of the solution. A rigid sample preparation method has to be followed in order to obtain reproducible spectra. About 15 mg of compound were dissolved in about 0.6 ml of $CDCl_3$ with about 6–7 drop of CD_3OD, then the solution was evaporated. The purpose of this step was to pre-exchange all the hydroxyl protons with deuterons. The sample was then dissolved in about 0.6 ml of $CDCl_3$ with about three drops of CD_3OD. The same sample was used to obtain all carbon, proton and 2D spectra.

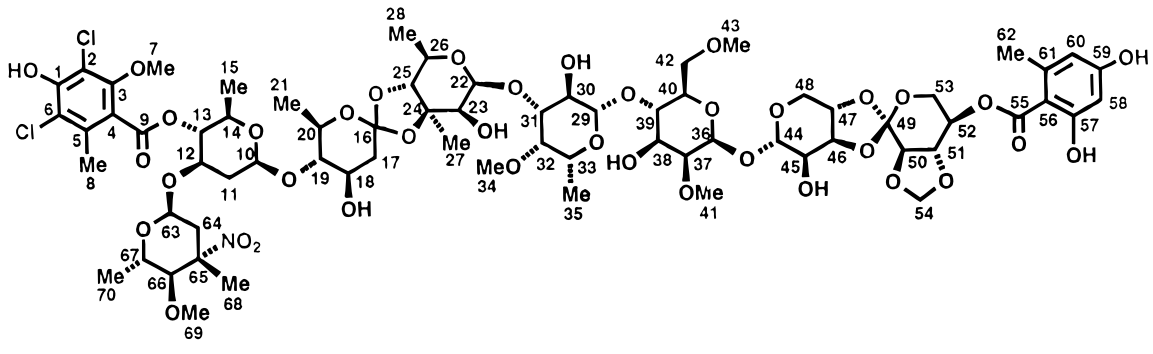

Figure 1. Structure of Sch 27899.

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Table 1. NMR spectral data for Sch 27899

Atom	^{13}C, δ (ppm)	^{1}H, δ (ppm)	Multiplicity, J (Hz)
1	151.24		
2	113.71		
3	153.53		
4	118.28		
5	134.71		
6	121.16		
7	62.02	3.88	s
8	17.99	2.38	s
9	165.86		
10	100.71	4.54	dd 1.5, 10
11	36.04	1.76	dt 10, 12, 12
		2.35	m
12	72.14	3.92	m
13	75.55	4.93	t 9, 9
14	71.30	3.59	m
15	18.09	1.43	d 6.5
16	120.40		
17	39.79	1.81	t 12.5, 12.5
		2.41	dd 12.5, 5
18	68.16	3.93	m
19	88.18	3.07	t 9, 9
20	70.05	3.84	m
21	17.77	1.25	d 6.5
22	101.02	5.03	d 1
23	72.95	4.08	d 1
24	80.50		
25	78.45	3.93	m
26	68.80	3.82	m
27	18.80	1.35	s
28	18.46	1.34	d 6
29	104.35	4.21	d 7.5
30	70.03	3.65	m
31	82.72	3.59	m
32	81.14	3.46	m
33	71.22	3.68	m
34	62.06	3.61	s
35	16.20	1.33	d 6.5
36	96.38	4.76	s
37	79.60	3.60	m
38	72.72	3.61	m
39	80.68	3.62	m
40	73.90	3.46	m
41	62.12	3.62	s
42	72.39	3.67, 3.81	m, m
43	59.18	3.39	s
44	97.74	5.23	d 1.5
45	68.91	4.45	m
46	80.93	3.97	m
47	69.12	4.43	dt 4.5, 10.5, 10.5
48	63.51	3.85	m
		4.17	dd 4.5, 9.5
49	119.19		
50	75.32	3.63	m
51	77.57	4.04	t 9.5, 9.5
52	70.98	5.41	dt 5.5, 9.5, 9
53	63.95	3.75	dd 9, 11.5
		4.26	dd 5.5, 11.5
54	97.05	5.15	s
		5.20	s
55	170.77		
56	103.83		
57	165.50		
58	101.09	6.25	d 2.5
59	162.77		
60	112.31	6.24	d 2.5
61	143.93		
62	24.56	2.47	s
63	92.59	4.99	dd 2, 5
64	40.07	2.04	dd 2, 13.5
		2.47	dd 5, 13.5
65	90.06		
66	84.41	3.65	m
67	66.46	3.49	m
68	19.46	1.68	s
69	60.82	3.36	s
70	17.77	0.87	d 6.5

NMR methods

^{1}H and ^{13}C NMR spectra were obtained on a Varian XL-400 spectrometer operating at a proton frequency of 400 MHz. All spectra were taken at 25 °C. The proton-detected 2D experiments, HMQC,[5,6] HMQC-TOCSY[7] and HMBC,[8] were performed on a Bruker/GE Omega-400 PSG spectrometer equipped with a 5 mm indirect detection probe from Nalorac (Martinez, CA, USA). All these experiments were performed either with standard pulse sequences supplied by the spectrometer manufacturer or with slightly modified pulse sequences. The 2D raw data were processed with Felix (Biosym/MSI). The number of data points for the HMQC and HMQC-TOCSY spectra was 512 × 512, and was 1K × 1K after processing. The spectral window in the carbon dimension was 12 500 Hz. Linear prediction was used to extend the raw data from 256 to 400 complex points in this dimension (t_1). Since the HMQC/HMQC-TOCSY spectra did not have enough resolution to yield accurate chemical shifts, these spectra were used to identify the relative positions of the resonances for assignment purpose only. The carbon chemical shifts in Table 1 were taken from the 1D carbon spectrum. For the HMBC spectra, the number of data points was 1K × 1K, and was 2K × 2K after processing. The spectral window in the carbon dimension was 17 241 Hz. The HMBC spectra were used mainly to assign the resonances of the non-protonated carbons.

HMQC was used to establish one-bond carbon–proton connectivities. HMQC-TOCSY at two different mixing times (15 and 30 ms) was used to establish proton–proton connectivities. The HMQC-TOCSY spectra at the two mixing times yielded information similar to the COSY and TOCSY spectra, respectively, but were more useful in crowded regions of the spectra since the correlations were separated in the second dimension by the chemical shifts of the carbon resonances. HMBC was used to establish long-range (two- or three-bond) carbon–proton connectivities. This experiment yielded complementary results to the HMQC, HMQC-TOCSY experiments by connecting the non-protonated carbons to the protons.

RESULTS AND DISCUSSION

The eight modified carbohydrate units are unique in structure and stereochemistry. After the networks of

proton and carbon resonances are established by the HMQC, HMQC-TOCSY and HMBC experiments, these networks can be unequivocally assigned to the individual carbohydrate rings. The proton and carbon resonances assignments are listed in Table 1. The experiments also firmly establish the carbon–proton skeletons of the molecules, since the connectivities of all the proton and carbon nuclei are determined.

For each of the eight modified carbohydrate rings, there is at least one proton resonance that is well resolved. Namely, they are the anomeric protons (H-10, H-22, H-29, H-36, H-44 and H-63), the three pairs of methylene protons (H-11, H-17 and H-64) and the methylenedioxy group (H-54). Starting from one assigned proton–carbon pair obtained from the HMQC spectrum, networks of protonated carbons can be readily assigned by using the HMQC-TOCSY spectra at two mixing times. Redundant information of each network are obtained at several carbon chemical shifts. To illustrate this assignment procedure, the modified carbohydrate ring C-10–C-15 is used as example. C-10 (100.71 ppm) is correlated to the anomeric proton H-10 (4.54 ppm) in the HMQC spectrum. In the HMQC-TOCSY spectrum with a short mixing time of 15 ms, C-10 correlates with H-10 and a pair of methylene protons, H-11 (1.76 and 2.35 ppm). When the mixing time is increased to 30 ms, C-10 correlates with H-10, H-11 and H-12 (3.92 ppm). C-11 (36.04 ppm) correlates with H-11, H-10, H-12 and H-13 (4.93 ppm). The coupling information of the network (H-10–H-15) is obtained at each of the six carbon chemical shift positions (C-10–C-15). The network starts with an anomeric proton (H-10) which is a doublet of doublets and ends with a methyl doublet (H-15). This network of protons and carbons can therefore unequivocally be assigned to the carbohydrate ring C-10–C-15. These assignments can also be confirmed by the HMBC spectrum; for example, C-13 correlates with H-12 and H-14 (two bonds) and with H-11 and H-15 (three bonds).

For the carbohydrate ring C-16–C-21, the proton network starts with a methylene proton pair (H-17), which connects sequentially to H-18, H-19, H-20 and ends with a methyl doublet (H-21). The corresponding carbon resonances are established by the HMQC and HMQC-TOCSY spectra. The network is connected to C-16 by the HMBC experiment.

For the carbohydrate rings with non-protonated carbon(s), the HMBC spectrum is used to connect the protonated carbon fragments. For illustration, the modified carbohydrate ring C-63–C-70 is used. C-70/H-70 (17.77/0.87 ppm) are connected to C-67/H-67 (66.46/3.49 ppm) and C-66/H-66 (84.41/3.65 ppm); C-63/H-63 (92.59/4.99 ppm) are connected to C-64/H-64 (40.07/2.04, 2.47 ppm) using the HMQC and HMQC-TOCSY spectra. In the HMBC spectrum, H-68 (1.68 ppm) is correlated with C-65 (90.06 ppm), C-64 and C-66; H-69 (3.36 ppm) is correlated only with C-66; H-70 (0.87 ppm) is correlated with C-67 and C-66.

The carbohydrate rings C-29–C-35, C-36–C-43 and C-44–C-48 can be assigned using a strategy similar to that used for the ring C-10–C-15. Rings C-22–C-28 and C-49–C-53 can be assigned in a way similar to ring C-63–C-70.

The sequence of the carbohydrate rings is also confirmed by the HMBC spectrum. Connection between carbohydrate rings C-63–C-70 and C-10–C-15 is supported by the correlations H-63 with C-12 and H-12 with C-63 in the HMBC spectrum. Similarly, the corre-

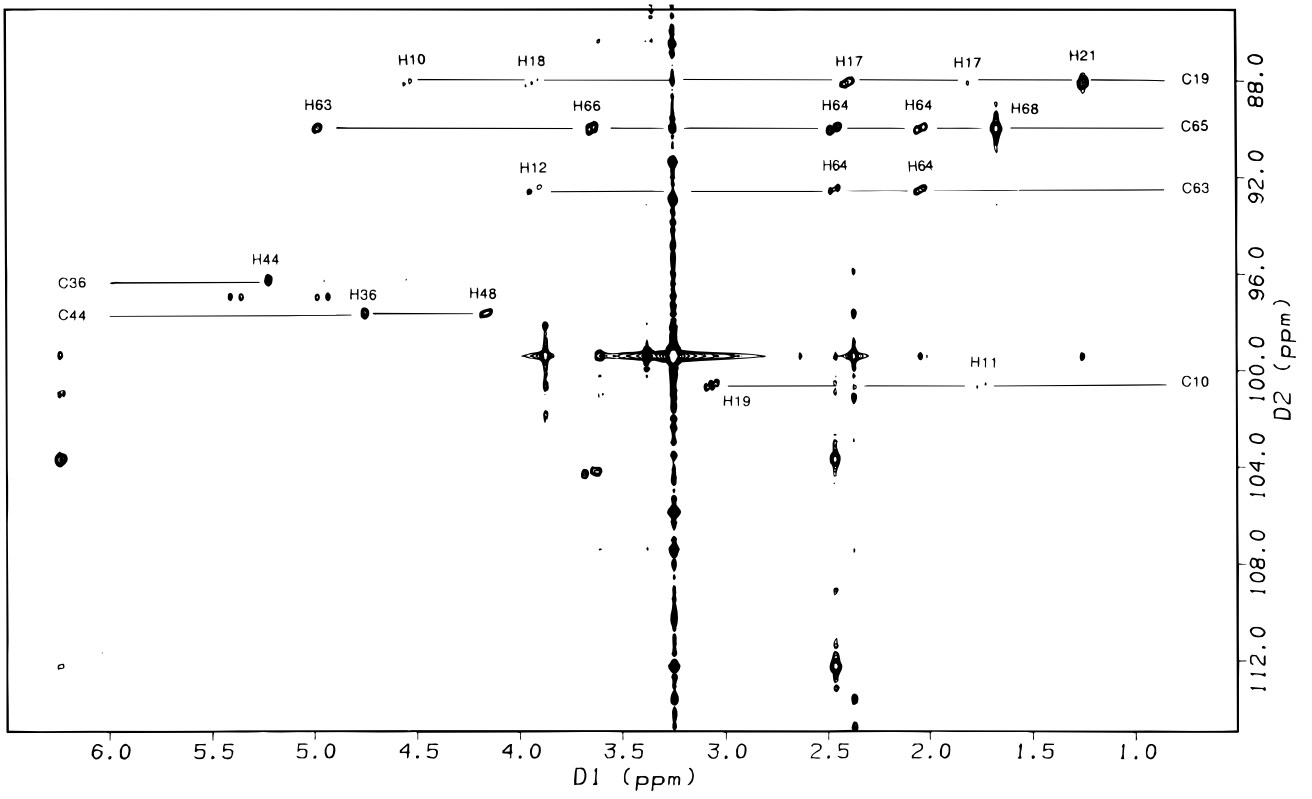

Figure 2. Part of the HMBC spectrum of Sch 27899, showing the region between 85 and 115 ppm in the ^{13}C dimension.

lations H-10 with C-19 and H-19 with C-10 establish the connection between rings C-10–C-15 and C-16–C-21. The inter-ring correlations C-31/H-22, C-29/H-39, C-39/H-29, C-36/H-44 and C-44/H-36 are also observed. Several of these correlations; C-19/H-10, C-10/H-19, C-63/H-12, C-36/H-44 and C-44/H-36, are shown in Fig. 2. The positions of the aromatic rings are confirmed by the correlations H-13/C-9 and H-52/C-55 observed in the HMBC spectrum.

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