QUINOLIDOMICINS A_1, A_2 AND B_1, NOVEL 60-MEMBERED MACROLIDE ANTIBIOTICS

II. STRUCTURE ELUCIDATION

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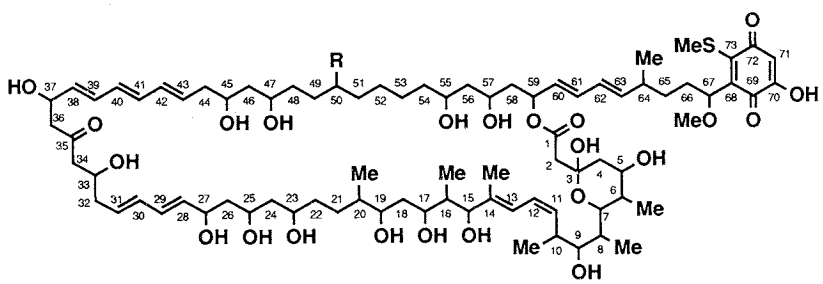
The structures of novel macrolide antibiotics, quinolidomicins A_1, A_2 and B_1, were elucidated as shown in Fig. 1 by NMR spectral analysis including a variety of two-dimensional techniques. The quinolidomicins possess a novel 60-membered macrolide ring, which is to our knowledge the largest among natural products.

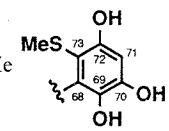
In the preceding paper[1], we have described the fermentation, isolation, physico-chemical properties and biological activity of new macrolide antibiotics, quinolidomicins A_1[2], A_2 and B_1, as well as the taxonomy of the producing organism, *Micromonospora* sp. JY16. This paper describes the structure elucidation of the quinolidomicins (Fig. 1).

The ^{13}C NMR spectrum of quinolidomicin A_1 (**1**) revealed the presence of 83 carbons, which were assigned to 10 methyls, 22 methylenes, 42 methines and 9 quaternary carbons by DEPT experiments. The heteronuclear single-quantum coherency (HSQC)[3] spectrum established all one-bond ^1H-^{13}C connectivities (Tables 1 and 2).

Fig. 1. Structures of quinolidomicins A_1 (**1**), A_2 (**2**) and B_1 (**3**).



Quinolidomicin A_1 (**1**) R=Me
Quinolidomicin B_1 (**3**) R=H



Quinolidomicin A_2 (**2**) R=Me

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Table 1. ^{1}H NMR data for quinolidomicin A_1 (**1**) in CD_3OD.

No.	δ_H	No.	δ_H	No.	δ_H
2	2.66, 2.61	29	6.21	55	3.75
4	2.10, 1.58	30	6.08	56	1.57
5	3.49	31	5.67	57	3.83
6	1.35	32	2.28, 2.22	58	1.87, 1.62
7	3.63	33	4.10	59	5.46
8	1.70	34	2.58	60	5.54
9	3.43	36	2.69, 2.64	61	6.22
10	3.00	37	4.57	62	5.98
11	5.52	38	5.67	63	5.57
12	6.27	39	6.24	64	2.16
13	6.42	40	6.12	65	1.45, 1.20
15	4.10	41	6.22	66	1.97, 1.75
16	1.77	42	6.13	67	4.78
17	3.90	43	5.75	71	5.66
18	1.62, 1.58	44	2.29	6-Me	0.81
19	3.55	45	3.82	8-Me	0.95
20	1.51	46	1.58, 1.49	10-Me	1.02
21	1.50, 1.24	47	3.71	14-Me	1.71
22	1.50	48	1.40	16-Me	0.93
23	3.72	49	1.41, 1.13	20-Me	0.90
24	1.58	50	1.40	50-Me	0.88
25	3.85	51	1.33, 1.13	64-Me	0.98
26	1.68, 1.59	52	1.42, 1.30	67-OMe	3.21
27	4.28	53	1.32	73-SMe	2.58
28	5.58	54	1.43		

A phase-sensitive double-quantum-filtered COSY (DQF-COSY)[4] experiment presented proton spin networks as shown in Fig. 2. Connectivities through overlapping proton signals were confirmed by ^{1}H-^{13}C long-range couplings observed in the heteronuclear multiple-bond correlation (HMBC)[5] spectrum. These analyses established partial structures **5** and **6** (Fig. 2).

Signals due to two quinone carbonyls (δ 182.6 and 181.0) and four aromatic carbons (δ 154.0, 150.7, 140.0 and 101.1) indicated the presence of a benzoquinone moiety. Both of a singlet aromatic proton (δ 5.66, 71-H) and an oxymethine proton (δ 4.78, 67-H) displayed long-range correlations to the carbonyl carbon (δ 181.0, C-69) and the aromatic quaternary carbon (δ 154.0, C-73), the latter of which was also coupled to a singlet methyl proton assigned to an *S*-methyl on the basis of its chemical shifts (δ_H 2.58, δ_C 19.2). Among the remaining two aromatic quaternary carbons (δ 150.7 and 140.0), one at a lower field (C-70) was identified as a phenolic carbon by its chemical shift and by the presence of an exchangeable proton at δ 7.00 in DMSO-d_6. The other (C-68) should be connected with the oxymethine carbon (C-67) substituted with a methoxy group (δ_H 3.21) as evidenced by their ^{1}H-^{13}C long-range coupling. These NMR data and a UV absorption maximum at 365 nm established a 2-methylthio-5-hydroxybenzoquinone chromophore. The proton spin system from 54-H to 67-H and the above results generated partial structure **4** as shown in Fig. 2.

Partial structure **7** was constructed by ^{13}C-^{1}H long-range correlations between a ketone carbonyl (δ 210.1, C-35) and the two terminal methylenes (34-H and 36-H) of the proton spin networks from 25-H and 40-H.

A partial structure representing C-4 to C-20 was established by ^{1}H-^{1}H connectivities and ^{1}H-^{13}C long-range couplings from six methyl groups to each three relevant carbons. An isolated methylene (δ 2.66

Table 2. ^{13}C NMR data for quinolidomicins A_1 (**1**), A_2 (**2**) and (**3**) in CD_3OD.

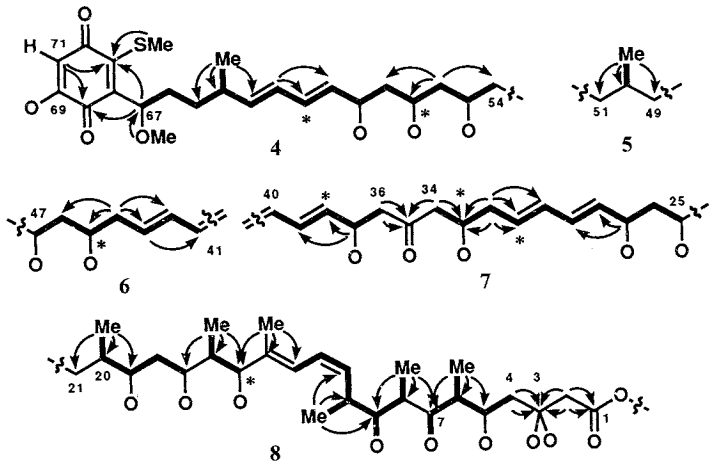
No.	1	2	3	No.	1	2	3
1	171.7 s	171.7 s	171.7 s	43	132.0 d	132.0 d	132.0 d
2	47.8 t	47.8 t	47.8 t	44	41.9 t	41.9 t	41.9 t
3	97.5 s	97.5 s	97.4 s	45	71.3 d	71.3 d	71.3 d
4	43.9 t	43.9 t	44.0[a] t	46	43.9 t	43.9 t	43.8[a] t
5	70.4 d	70.5 d	70.4 d	47	71.6 d	71.6 d	71.4 d
6	41.3 d	41.4 d	41.3 d	48	35.9 t	35.9 t	38.5 t
7	75.4 d	75.4 d	75.4 d	49	33.4 t	33.5 t	26.5[b] t
8	38.0 d	38.0 d	38.0 d	50	33.8 d	33.9 d	30.6 t
9	79.0 d	79.0 d	79.0 d	51	38.0 t	38.0 t	30.6 t
10	35.2 d	35.3 d	35.2 d	52	26.8 t	26.8 t	30.6 t
11	134.2 d	134.2 d	134.1 d	53	28.1 t	28.1 t	26.4[b] t
12	125.0 d	125.1 d	125.0 d	54	38.7 t	38.7 t	38.5 t
13	122.9 d	122.9 d	122.9 d	55	71.4 d	71.4 d	71.4 d
14	140.0 s	140.0 s	140.0 s	56	45.3 t	45.3 t	45.4 t
15	81.0 d	81.0 d	81.0 d	57	67.8 d	67.8 d	67.8 d
16	41.2 d	41.2 d	41.2 d	58	43.5 t	43.6 d	43.6 d
17	73.5 d	73.6 d	73.5 d	59	73.5 d	73.6 d	73.5 d
18	38.3 t	38.3 t	38.3 t	60	130.2 d	130.2 d	130.2 d
19	75.7 d	75.7 d	75.7 d	61	134.5 d	134.5 d	134.5 d
20	40.4 d	40.5 d	40.5 d	62	129.4 d	129.4 d	129.4 d
21	28.8 t	28.8 t	28.8 t	63	142.7 d	142.9 d	142.7 d
22	36.4 t	36.4 t	36.4 t	64	38.1 d	38.1 d	38.1 d
23	71.4 d	71.4 d	71.4 d	65	34.3 t	34.2[c] t	34.3 t
24	45.4 t	45.4 t	45.4 t	66	31.7 t	34.5[c] t	31.7 t
25	69.7 d	69.7 d	69.7 d	67	80.1 d	85.2 d	80.1 d
26	45.6 t	45.6 t	45.6 t	68	140.0 s	—*	140.0 s
27	71.7 d	71.7 d	71.7 d	69	181.0 s	—	181.0 s
28	135.6 d	135.6 d	135.6 d	70	150.7 s	—	150.7 s
29	131.9 d	131.9 d	131.8 d	71	101.1 d	102.4 d	101.1 d
30	133.9 d	133.9 d	133.9 d	72	182.6 s	—	182.6 s
31	131.1 d	131.1 d	131.1 d	73	154.0 s	—	154.0 s
32	41.5 t	41.6 t	41.5 t	6-Me	12.3 q	12.3 q	12.3 q
33	68.5 d	68.5 d	68.5 d	8-Me	9.2 q	9.3 q	9.2 q
34	51.2 t	51.2 t	51.2 t	10-Me	19.0 q	19.0 q	19.0 q
35	210.1 s	210.1 s	210.1 s	14-Me	12.7 q	12.7 q	12.7 q
36	51.8 t	51.8 t	51.8 t	16-Me	8.1 q	8.2 q	8.1 q
37	69.3 d	69.3 d	69.3 d	20-Me	15.6 q	15.6 q	15.6 q
38	135.7 d	135.7 d	135.7 d	50-Me	20.3 q	20.3 q	
39	131.7 d	131.7 d	131.7 d	64-Me	21.0 q	21.1 q	21.0 q
40	131.7 d	131.7 d	131.7 d	67-OMe	57.4 q	57.9 q	57.4 q
41	134.5 d	134.5 d	134.5 d	73-SMe	19.2 q	20.0 q	19.2 q
42	134.2 d	134.2 d	134.1 d				

[a~c] Interchangeable.
* Signals not observed.

and 2.61, 2-H) revealed ^1H-^{13}C long-range correlations to an ester carbonyl (δ 171.7, C-1) and a ketal carbon (δ 97.5, C-3), the latter was also coupled to the terminal methylene (δ 2.10 and 1.58, 4-H) of the above partial structure. These data yielded partial structure **8** as shown in Fig. 2.

The protons on C-4 to C-7 in **8** showed large vicinal-coupling constants ($J_{4ax\text{-}5}=10.5$ Hz, $J_{5\text{-}6}=10.5$ Hz and $J_{6\text{-}7}=10.0$ Hz) and were required to be in a six-membered ring, thereby showing that C-3 was connected to C-7 with an ether linkage. The characteristic UV absorptions at λ_{max} 257, 268 and 279 nm indicated the presence of a triene group[6], which could be formed by only connection between C-40 in **7** and C-41 in **6**.

Fig. 2. Partial structures of quinolidomicin A_1 (**1**).



Bold lines show proton spin networks obtained by a DQF-COSY experiment. Arrows show ^1H-^{13}C long-range correlations. Asterisks show unresolved protons.

A partial structure including C-47 to C-55 presented the most difficult part of the structure determination because of the severe overlap of the methylene signals in the ^1H NMR spectrum, and was elucidated by a phase-sensitive HSQC-homonuclear Hartmann-Hahn (HSQC-HOHAHA)[3,7] experiment, which enables one to trace proton spin networks by observing relevant well-separated ^{13}C-signals. The spectrum (Fig. 3) showed relayed connectivities from 47-H to C-48 and C-49, from 55-H to C-54, C-53 and C-52, and from 50-CH_3 to C-48, C-49, C-50, C-51 and C-52, thereby showing the sequence of C-47 to C-55 between **4** and **6** through **5**, as shown in Fig. 4. The sequence of C-21 to C-25 present between **7** and **8** was also assigned on the basis of the relay from 23-H to C-21, C-22, C-24 and C-25 (not shown), and from 25-H to C-22, C-23 (not shown) and C-24 (Figs. 3 and 4).

The correlation between a broad multiplet oxymethine proton (δ 5.46, 59-H) and an ester carbonyl carbon (δ 171.7, C-1), which could not be observed by HMBC experiments, was revealed by a new technique using phase-sensitive ^{13}C-decoupled HMBC (D-HMBC, Fig. 5)[2,8,9] to generate a 60-membered lactone ring.

The geometrical configurations of the olefinic bonds were established to be 11*Z*, 28*E*, 30*E*, 38*E*, 42*E*, 60*E* and 62*E* by their vicinal coupling constants ($J_{11\text{-}12}=11.5$ Hz, $J_{28\text{-}29}=15.5$ Hz, $J_{30\text{-}31}=15.0$ Hz, $J_{38\text{-}39}=15.5$ Hz, $J_{42\text{-}43}=15.0$ Hz, $J_{60\text{-}61}=15.0$ Hz and $J_{62\text{-}63}=15.0$ Hz). The stereochemistry at C-13 was determined to be *E* on the basis of a high-field chemical shift for 14-CH_3 (δ_C 12.7) and an NOE observed between 12-H and 14-CH_3. The remaining olefinic bond (C-40) in the triene system was required to have an *E* configuration by the ^{13}C chemical shift similarity of C-38 to C-43 to those of the all-*E* triene moiety (δ 132.2, 137.8, 130.7, 135.9, 133.5, and 133.5) of pulvomycin[10]. Thus, the planar structure of quinolidomicin A_1 (**1**) was established as shown in Fig. 1.

The molecular formula $C_{83}H_{134}O_{23}S$ of quinolidomicins A_2 (**2**) showed that **2** is a dihydro derivative of **1**. The ^{13}C chemical shifts of C-1 to C-65 of **2** were almost identical with those of **1**, and downfield shifts were observed with signals due to C-66, C-67, C-71 and *S*-CH_3 (Table 2). Although quaternary carbons in the aromatic chromophore of **2** were not observed by extensive line broadening, the molecular

Fig. 3. Phase-sensitive HSQC-HOHAHA spectra of quinolidomicin A_1 (**1**).

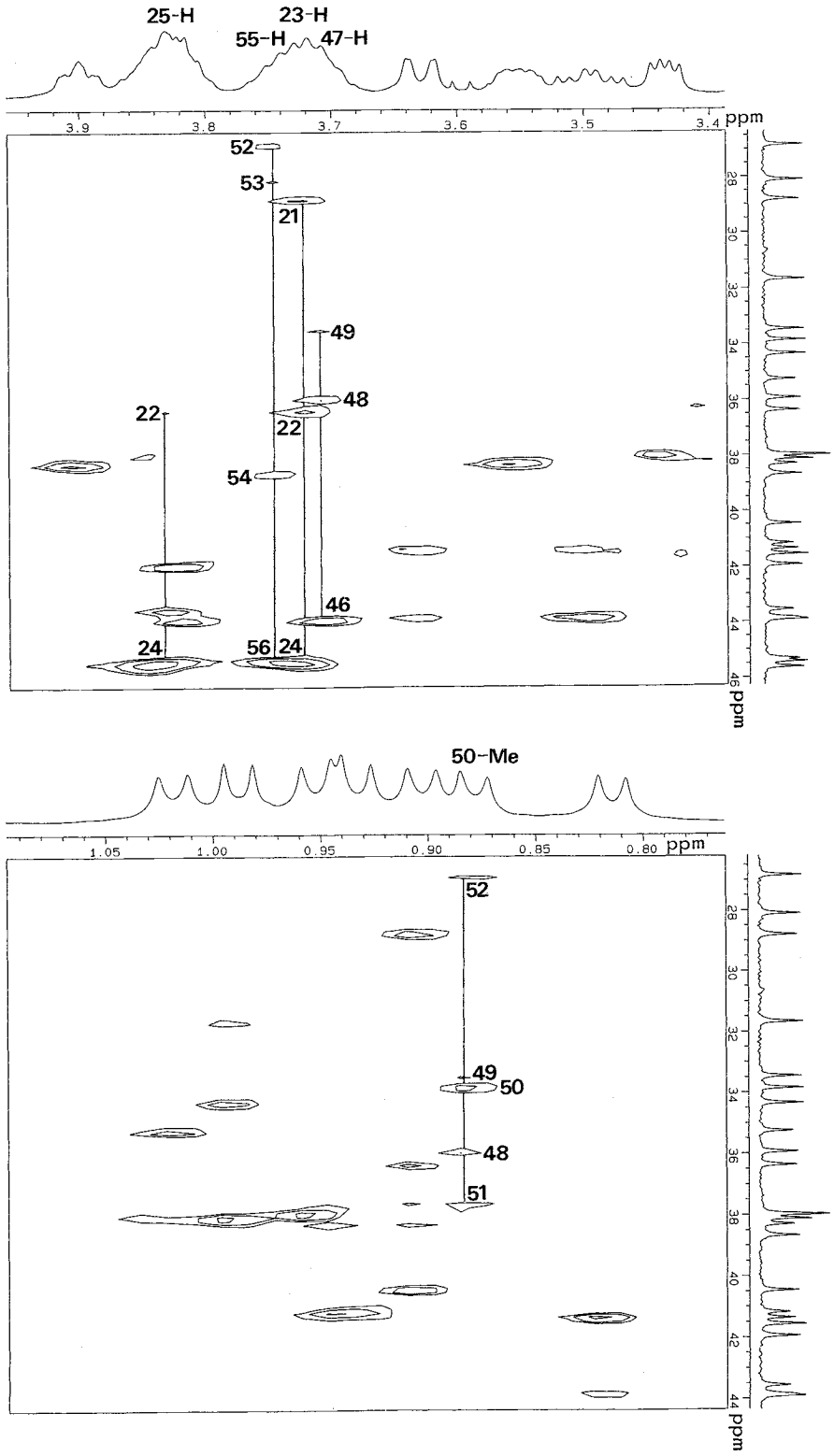
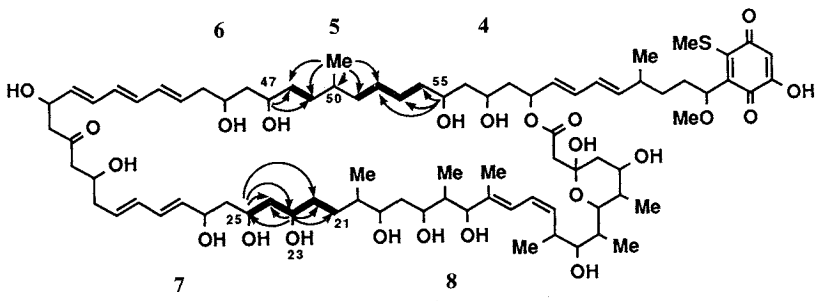
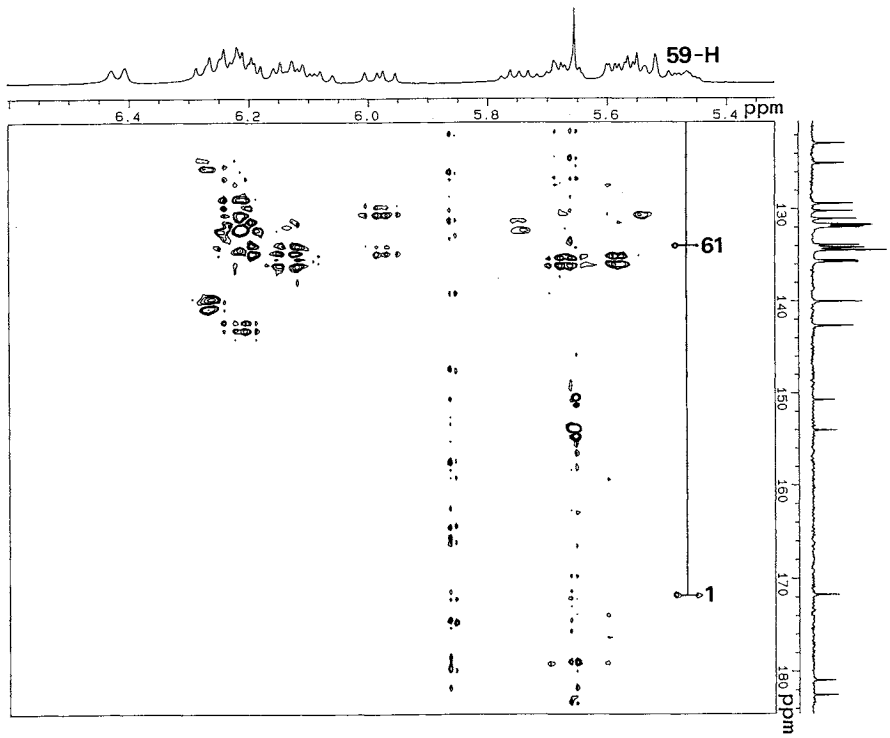


Fig. 4. HSQC-HOHAHA data summary for quinolidomicin A_1 (**1**).



Arrows show ^{1}H-^{13}C relayed connectivities.

Fig. 5. D-HMBC spectrum of quinolidomicin A_1 (**1**).



formula and the conversion of **2** to **1** by air oxidation revealed that **2** possesses a hydroquinone moiety in place of the benzoquinone chromophore of **1**. Thus, the structure of **2** was determined as shown in Fig. 1.

Quinolidomicin B_1 (**3**) showed similar ^{1}H and ^{13}C NMR spectra to those of **1** except for 50-CH_3. In the ^{13}C NMR spectra of **3**, 50-CH_3 disappeared and a methine carbon (C-50) of **1** was replaced by a methylene carbon (δ 30.6), followed by upfield shifts for C-49, C-51 and C-53 and downfield shifts for C-48 and C-52 (Table 2). The ^{13}C chemical shifts for the other parts of **3** were almost the same as those of **1**. These results and the molecular formula $C_{82}H_{130}O_{23}S$ established the structure of **3** as the 50-demethyl derivative of **1** as shown in Fig. 1.

The quinolidomicins represent a new class of macrolides containing a benzoquinone chromophore and a 60-membered lactone ring, which is far larger than that present in monazomycin[11], a compound known to have the largest ring in natural products.

Experimental

NMR spectra were obtained on a JEOL JNM-A500 spectrometer with ^1H NMR at 500 MHz and with ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard. All NMR experiments for **1** were performed on 10 mg of **1** dissolved in 0.8 ml of CD_3OD at 27°C.

The phase-sensitive HSQC spectrum was obtained from a 512×512 data matrix with 64 scans per t_1 value. The spectral widths were 17,700 Hz for carbon and 3,170 Hz for proton. The pulse repetition time was 1.5 seconds, and accumulation of data required 14 hours.

The phase-sensitive DQF-COSY spectrum resulted from a $512 \times 1,024$ ($t_1 \times t_2$) data matrix. The spectral width was 3,245 Hz in both dimensions. Sixty-four scans were recorded for each t_1 value with a pulse repetition time of 1.5 seconds. The total measuring time was 14 hours.

The HMBC spectrum resulted from a 256×512 ($t_1 \times t_2$) data matrix, with 160 scans per t_1 value. The spectral widths were 26,600 Hz for carbon and 3,240 Hz for proton. A pulse repetition time of 1.2 seconds was used, and accumulation of data required 16 hours.

The phase-sensitive HSQC-HOHAHA spectrum was obtained from a $512 \times 1,024$ ($t_1 \times t_2$) data matrix. The spectral widths were 17,700 Hz and 3,170 Hz in the t_1 and t_2 dimensions, respectively. A mixing time of 60 mseconds was used. Eighty scans were recorded for each t_1 value with a pulse repetition tme of 1.5 seconds. The total measuring time was 16 hours.

The phase-sensitive D-HMBC spectrum resulted from a $256 \times 1,024$ ($t_1 \times t_2$) data matrix. The spectral widths were 26,600 Hz for carbon and 4,500 Hz for proton. A delay time of 120 mseconds was used. For each t_1 value, 96 scans were recorded with a pulse repetition time of 1 second. The total measuring time was 21 hours.

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