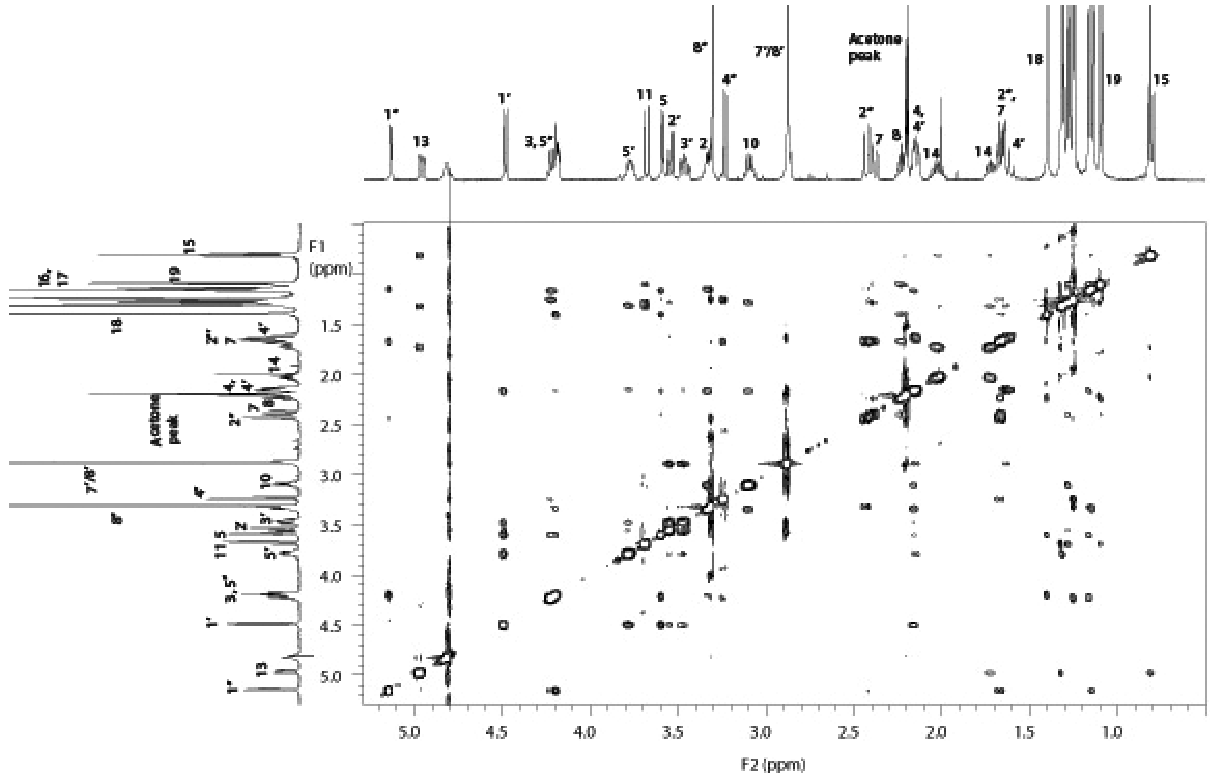

Figure 1. A contour plot of the 500 MHz ROESY spectrum of anhydroerythromycin A (1) in deuteriated phosphate buffer at apparent pH 7.0, 25 °C.

Table 1. 500 MHz ROESY connectivity table of anhydroerythromycin A (**1**) in deuteriated phosphate buffer at apparent pH 7.0, 25 °C; mixing time is 500 ms

	2	3	4	5	7r	7s	8	10	11	13	14s	14r	15	16	17	18	19	20	21	1'	2'	3'	4'r	4's	5'	6'	7',8'	1"	2"r	2"s	4"	5"	6"	7"	8"
2	●	S	M					M						M																					
3	S	●	V	S												M												M							
4	M	V	●	V				S							S					M															
5		S	V	●											M	M				M												M			
7r					●	L	S									S																			
7s					L	●	S										S	S																	
8					S	S	●									S	M																		
10	M		S					●										M																	
11									●								S	M	M																
13										●		M	M						M																
14s											●	L	V																						
14r										M	L	●	V						S																
15										M	V	V	●																						
16	M													●														M							
17			S	M											●																	M			S
18		M		M	S		S									●																			
19						S	M		S								●	S																	
20						S		M	M								S	●			V														
21									M	M		S							●																
1'			M	M																●	S	M			L										
2'																		V		S	●				V		M								
3'																				M		●		V	S		M								
4'r																							●	L		S	S								
4's																						V	L	●	S		S								
5'																				L	V	S		S	●	M									
6'																							S		M	●									
7',8'																					M	M	S	S			●								
1"		M												M														●	M	V		V			
2"r																												M	●	L	M				
2"s																												V	L	●					M
4"																													M		●	S		L	
5"				M											M													V			S	●	L		
6"																																L	●		
7"																															L			●	M
8"															S															M				M	●
	2	3	4	5	7r	7s	8	10	11	13	14s	14r	15	16	17	18	19	20	21	1'	2'	3'	4'r	4's	5'	6'	7',8'	1"	2"r	2"s	4"	5"	6"	7"	8"

Very small signal: V; Small signal : S; Medium Signal: M; Large signal: L

Chemical shifts of methylene protons

H-7r: δ 1.65; H-7s: δ 2.4; H-14s: δ 2.0; H-14r: δ 1.7; H-4'r: δ 1.62; H-4's: δ 2.18; H-2"r: δ 1.7; H-2"s: δ 2.43. The symbols r and s are used as abbreviations for *proR* and *pros* in this table.

Full NMR assignments of anhydroerythromycin A 2'-acetate (4) in $CDCl_3$

Anhydroerythromycin A 2'-acetate (**4**) was dissolved in $CDCl_3$ to a concentration of 30 mg ml^{-1}. In the 1D-^1H spectrum there were three readily recognizable singlets at δ 3.28, 2.26 and 2.10 integrating respectively to three, six and three protons. These were assigned by inspection to the methyl groups at 8'', 7'/8' and 10' respectively. Their corresponding carbon chemical shifts were found from the gHMQC spectrum. The only triplet in the upfield region, δ 0.83, was assigned to CH_3-15. C-15 was assigned from the gHMQC spectrum. In the gHMBC spectrum, H_3-15 coupled to δ 24.24 (a negative signal in DEPT-135 spectrum) and δ 81.35. These were consistent with C-14 and C-13, respectively. H_3-15 showed, in the DQF-COSY spectrum, correlation with H_2-14 at δ 1.66 and 2.04. Now the TOCSY spectrum was used to assign δ 5.17, a double doublet signal in 1D-^1H spectrum, to H-13. The spin group H-13 to H-14_s, H-14_r and H3-15 was very clear in the TOCSY spectrum. In the gHMBC spectrum H-13 demonstrated correlation with C-12 at δ 82.52 and also it was used to assign δ 1.29 to H_3-21, a singlet in the 1D-^1H spectrum, based on its correlation with C-12 and C-13. C-21 appeared at δ 25.19 in the gHMQC spectrum.

There were two remaining isolated singlets in the 1D-^1H spectrum at δ 1.21 and 1.42. These were respectively assigned to H_3-7'' and H_3-18. Their corresponding carbon chemical shifts were found through the gHMQC spectrum. C-18, in the gHMBC spectrum, showed connections with δ 3.43, 2.48 and 1.46. These were (non-specifically) H_2-7 and H-5, but were distinguished by the strong mutual coupling between δ 1.46 and 2.48 in the DQF-COSY spectrum. Now the gHMQC spectrum was used to assign C-7 and C-5. Both protons attached to C-7 coupled to H-8 at δ 2.28. C-8 was found from the gHMQC spectrum; in the gHMBC spectrum C-8, in addition to coupling to H_2-7, coupled to δ 1.07 which is consistent with H_3-19.

A new starting point was chosen from the DEPT-135 spectrum. There were two remaining methylene groups at δ 30.64 and 34.56 were assigned non-specifically to C-2'' and C-4'. In the gHMQC spectrum, δ 34.56 showed proton signals at δ 1.53 and 2.28 (overlapping H-8). In the TOCSY spectrum these two proton signals coupled to δ 5.13, a doublet in 1D-^1H spectrum. This spin group was comfortably consistent with H-$2''_s$, H-$2''_r$ and H-1''. Hence the remaining methylene group at δ 30.56 was assigned to CH_2-4'. Now the gHMQC spectrum was used to assign C-1'' and H-4'. In the TOCSY spectrum, the H-4' at δ 1.75 coupled to δ 1.24, 1.33, 2.71, 3.49, 4.34 and 4.83. These interactions demonstrated a clear spin group existing in the desosamine sugar. The most downfield chemical shift was assigned to H-2' that showed correlation, in the DQF-COSY spectrum, with H-1' (at δ 4.34) and H-3' (at δ 2.71). The DQF-COSY spectrum was used to assign H-5' (at δ 3.49) from its correlation with H-4' and subsequently H-6' was determined from its coupling with H-5'. C-1', C-2' and C-3' were assigned using the gHMQC spectrum. C-5' and C-6' were assigned through their connections to H-4' (at δ 1.75) in the gHMBC spectrum.

In the 1D-^1H spectrum, there were three overlapping methyl signals at δ 1.07 – 1.08. One of these had already been assigned to H_3-19. The TOCSY and DQF-COSY spectra were now used to assign the spin group H-5/H-4/H_3-17/H-3/H-2/H_3-16. The remaining 2 methyl groups at δ 1.07 – 1.08 could now be assigned to H_3-16 and H_3-17. The carbon chemical shifts for C-2, C-3, C-4, C-16 and C-17 were found through their corresponding proton chemical shifts in the gHMQC spectrum.

The remaining spin group in the TOCSY spectrum (δ 3.98, δ 3.02, 1.87 and 1.21) was assigned to the cladinose sugar, H-5", H-4", OH-4" and H_3-6" respectively. The OH-4" signal, a broad singlet, was missing in the DQF-COSY and gHMBC spectra. Now the gHMQC spectrum was used to assign C-4", C-5" and C-6". The chemical shift of C-3" was found through its connection to H-1" and H-2" in the gHMBC spectrum.

C-1 and C-9' were the most downfield signals in the 1D-^{13}C spectrum. The assignment of C-1 to δ 178.57 was confirmed by the presence in the gHMBC spectrum of a crosspeak to a high frequency double doublet at δ 5.17, characteristic of H-13. The C-1 signal in the gHMBC spectrum was in fact folded back at about δ 35.8. This C-9' quaternary signal was assigned at δ 170.10 and showed couplings to H-2' and H_3-10' in the gHMBC spectrum.

There was a quaternary signal in the 1D-^{13}C spectrum that demonstrated couplings to δ 2.47 (H-7), 1.94 (H-4) and 1.42 (H_3-18). This signal must be C-6. Therefore the remaining quaternary signal in the 1D-^{13}C spectrum at δ 116.03 was assigned to C-9 showing connections to H_3-19, H-8 and H-7 (at δ 1.46) in the gHMBC spectrum. In addition to these correlations, C-9 in the gHMBC spectrum also coupled to δ 2.97 and 1.31. The signal at δ 1.31 was an isolated doublet in the 1D-^{1}H spectrum integrated to three protons. This was consistent with H_3-20, the only remaining methyl group. The signal at δ 2.97, overlapping with H-2, was then assigned to H-10; this signal showed a correlation with H_3-20 in the DQF-COSY spectrum. H-10 also coupled to δ 3.50, assigned to H-11. A signal that appeared only in the TOCSY spectrum at δ 2.02, overlapping with H-14 in the 1D-^{1}H spectrum, coupled to H-10 and H-11, and was assigned to 11-OH. The completion of the assignments was carried out by assigning C-10 and C-11 through their appearance in the gHMQC spectrum. The full assignments are summarized in table 1.

Table 2. The full ^{1}H and ^{13}C NMR assignments of anhydroerythromycin A 2'-acetate in $CDCl_3$.

Positin	Multiplicity	^{1}H (ppm)	J_{HH} (Hz)	^{13}C (ppm)	HMBC Connectivities ($^{13}C \rightarrow ^{1}H$)
1	–	–	–	178.57	H(2), H(3), H_3(16), H(13)
2	m	2.96[b]	–	46.37	H_3(16)
3	dd	4.31	6.6, 3.9	76.34	H(4), H(5), H_3(16), H(1")
4	dq	1.94	13.2, 6.7	44.08	H(3), H(5), H_3(17)
5	d	3.43	5.5	85.58	H(4), H(7), H_3(17), H_3(18), H(1')
6	–	–	–	81.29	H(4), H(7), H_3(18)
7	dd	1.46	12.6, 6.6	41.26	H_3(18)
	brt	2.48	13.6		
8	obscured	2.28	–	41.28	H_3(19), H_2(7)
9	–	–	–	116.03	H(7), H(8), H(10), H_3(19), H_3(20)
10	m	2.97[b]	–	51.58	H_3(20)
11	obscured	3.50	–	87.23	H(10), H_3(20), H_3(21)
11-OH	obscured	2.04	-	-	-
12	–	–	–	82.58	H(13), H_3(21)
13	dd	5.17	11.7, 2.9	81.36	H(14), H_3(15), H_3(21)
14	dddd	1.66	14.1, 7.3, 2.9	24.24	H_3(15), H(13)
	m	2.04	–		
15	t	0.83	7.4	10.95	H_2(14)
16	d	1.07	6.6	14.75	H(2)
17	d	1.08	7.0	15.97	H(4), H(5)
18	s	1.42	–	28.56	H(5), H_2(7)
19	d	1.07	6.6	12.24	H(7), H(8)
20	d	1.31	7.3	14.03	H(10), H(11)
21	s	1.29	–	25.19	–
1'	d	4.34	7.7	100.80	H(5), H(2')
2'	dd	4.83	10.6, 7.6	71.14	H(3'), H(4')
3'	ddd	2.71	23.7, 12.4, 4.3	63.46	H(2') H_2(4'), H_3(7'/8')
4'	obscured	1.33	–	30.64	H_3(6')
	brdd	1.75	13.2, 2.6		
5'	m	3.49[a]	–	69.02	H(4'),H_3(6')
6'	d	1.24	6.0	21.04	H(4')
7'/8'	s	2.26	–	40.65	H(3'), H_3(7'/8')
9'	–	–	–	170.10	H(2'), H_3(10'),
10'	s	2.10	–	21.64	H(4')
1"	d	5.13	4.4	95.42	H(3), H_2(2")
2"	dd	1.53	15.2, 4.9	34.56	H_3(7")
	obscured	2.28	–		
3"	–	–	–	72.83	H(1"), H(2"), H_3(7"), H_3(8")
4"	d	3.02	9.9	78.23	H(2"), H(5"), H_3(6")
4"-OH	brs	1.87	-	-	-
5"	dq	3.98	9.3, 6.2	65.15	H(1"), H(4"), H_3(6")
6"	d	1.21[a]	7.7	17.75	H(4")
7"	s	1.21	–	21.63	H_3(17), H(2")
8"	s	3.28	–	49.33	–

a: partially obscured

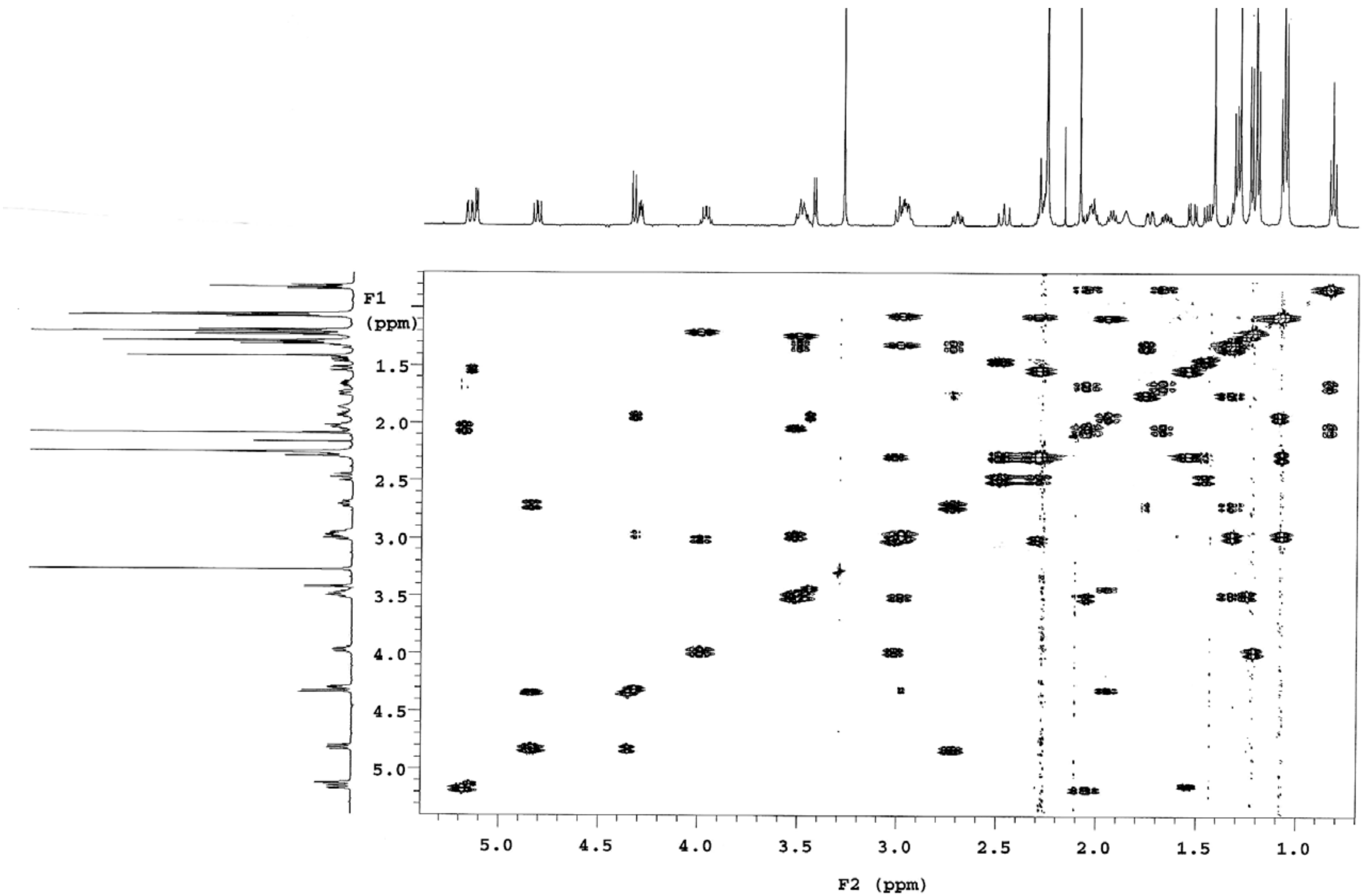

Figure 2. A contour plot of the 500 MHz DQF-COSY spectrum of anhydroerythromycin A 2'-acetate (**4**) in $CDCl_3$.

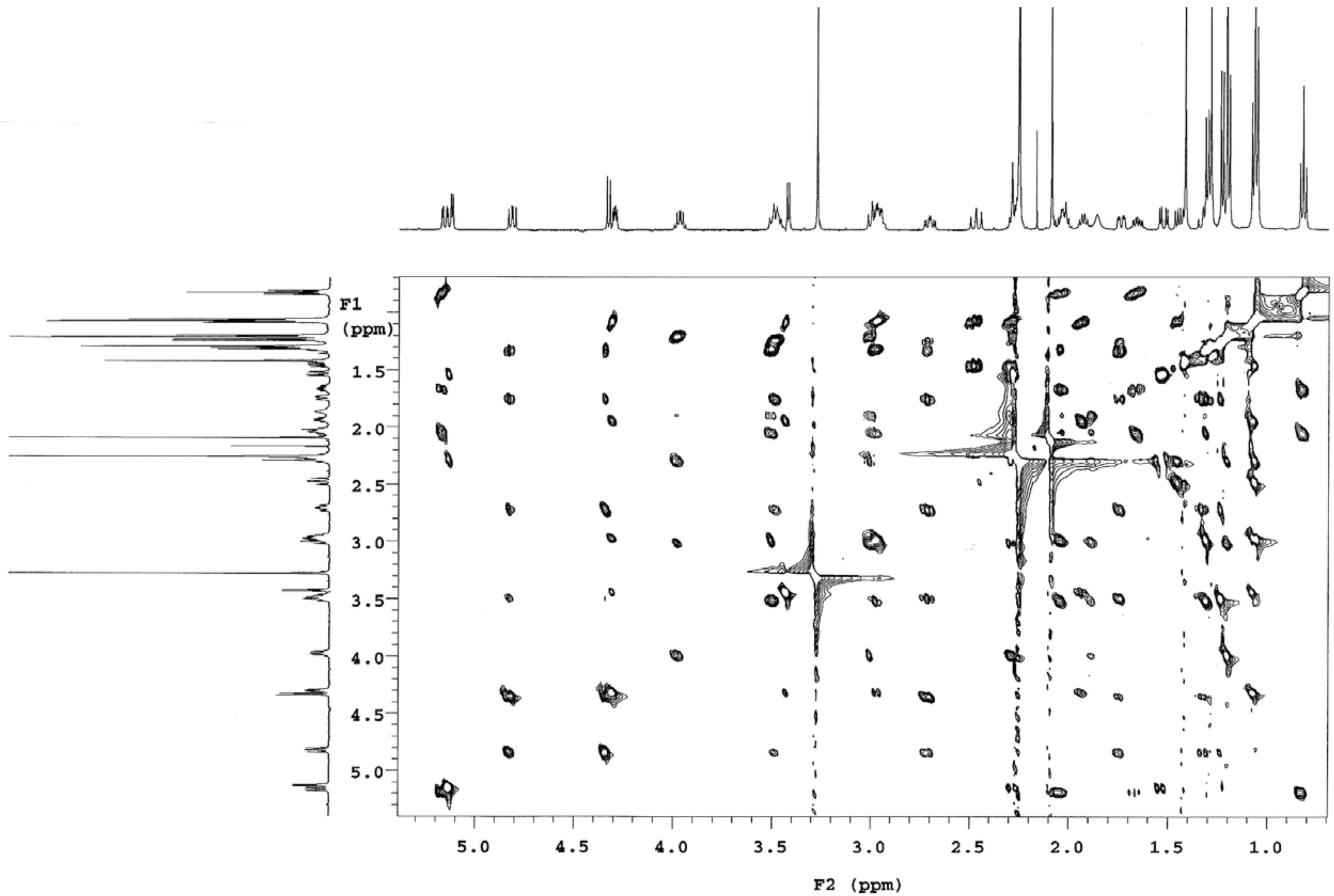

Figure 3. A contour plot of the 500 MHz TOCSY spectrum of anhydroerythromycin A 2'-acetate (**4**) in $CDCl_3$.

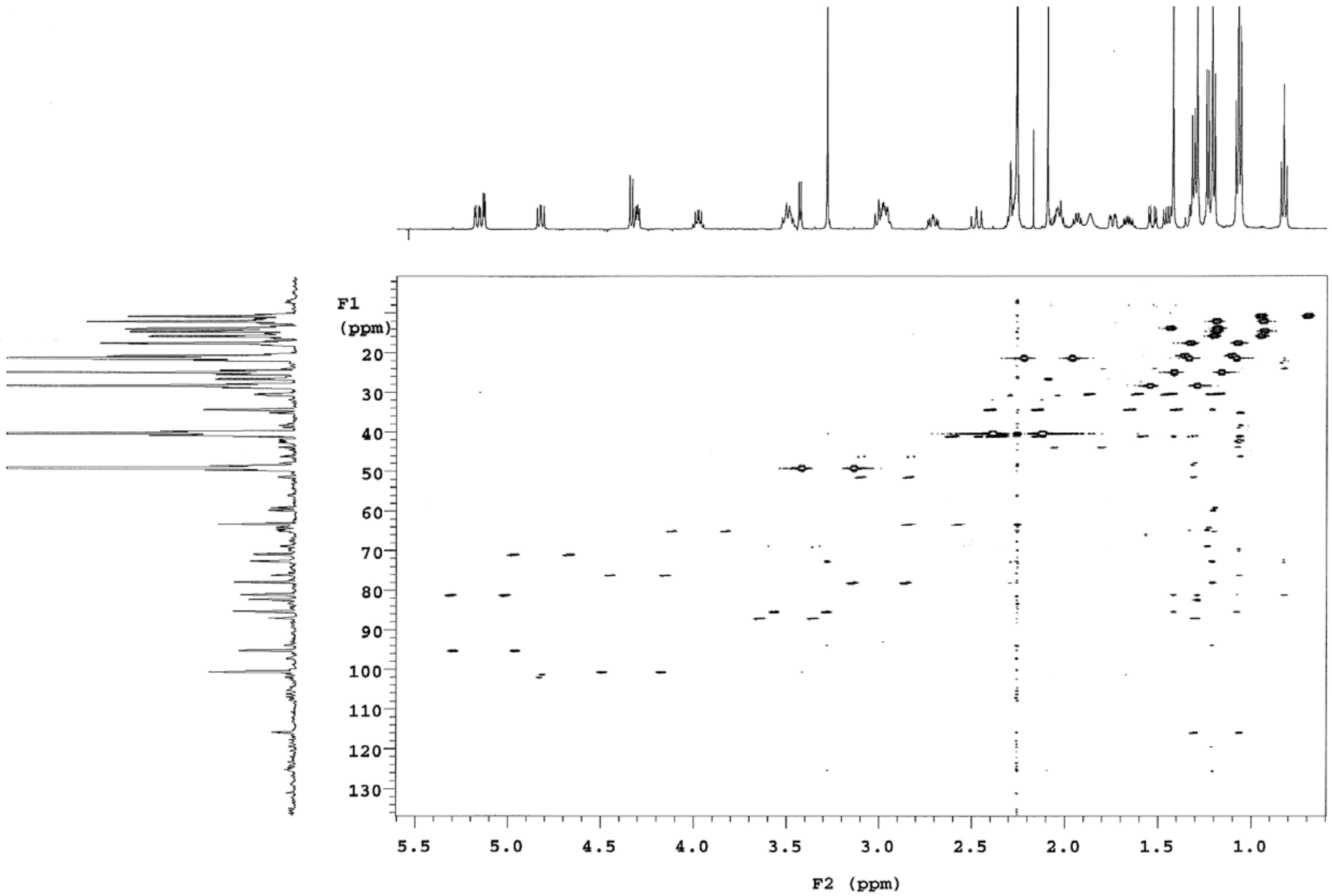

Figure 4. A contour plot of the 500 MHz gHMQC spectrum of anhydroerythromycin A 2'-acetate (**4**) in $CDCl_3$.

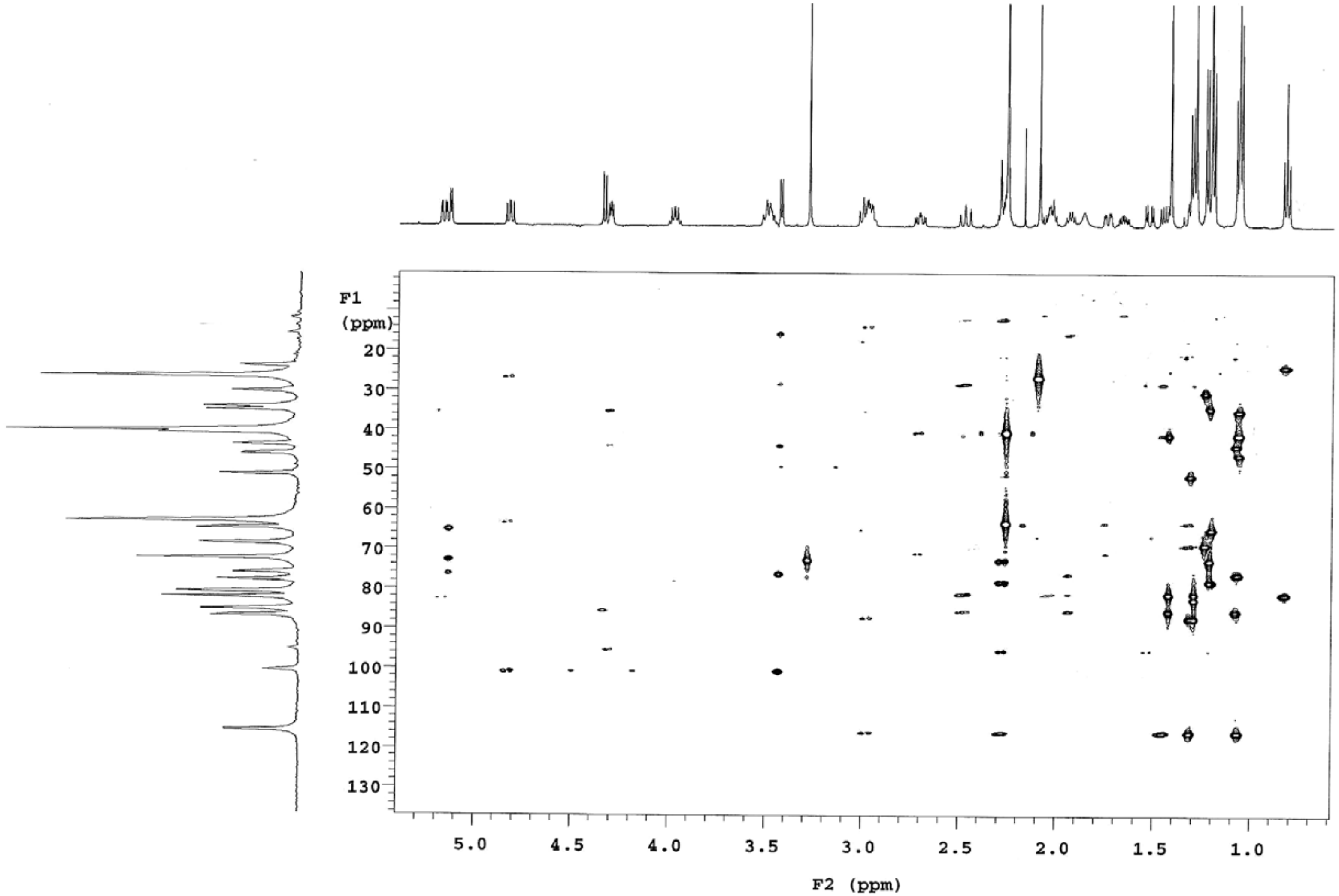

Figure 5. A contour plot of the 500 MHz GHMBC spectrum of anhydroerythromycin A 2'-acetate (**4**) in $CDCl_3$.