Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Biljana Arsic[a], Abida Awan[a], Richard J. Brennan[a], Juan A. Aguila[b], Andrew C. Regan[b], Ruth Ledder[a], Andrew J. McBain[a] and Jill Barber[a*]

[a]Manchester Pharmacy School, University of Manchester, Manchester UK, M13 9PT

[b]School of Chemistry, University of Manchester, Manchester UK, M13 9PL

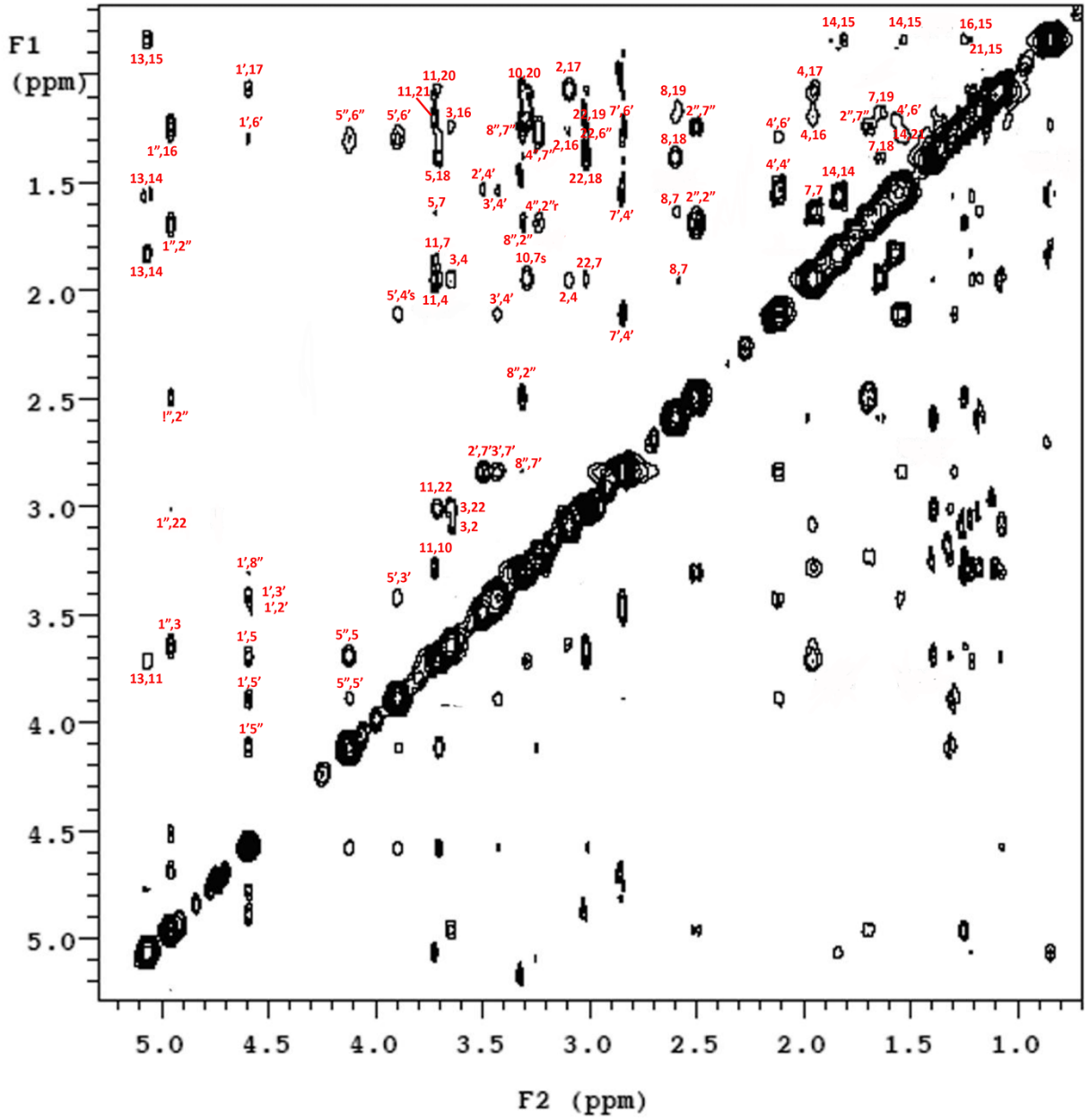

Fig S1A: 600 MHz TRNOESY NMR spectra of clarithromycin (4 mmol dm^{-3}) in the presence of 0.8 µmol dm^{-3} ribosomes in 50 mmol dm^{-3} sodium phosphate buffer in D_2O pD 7.6 (uncorrected value), mixing time 75 ms.

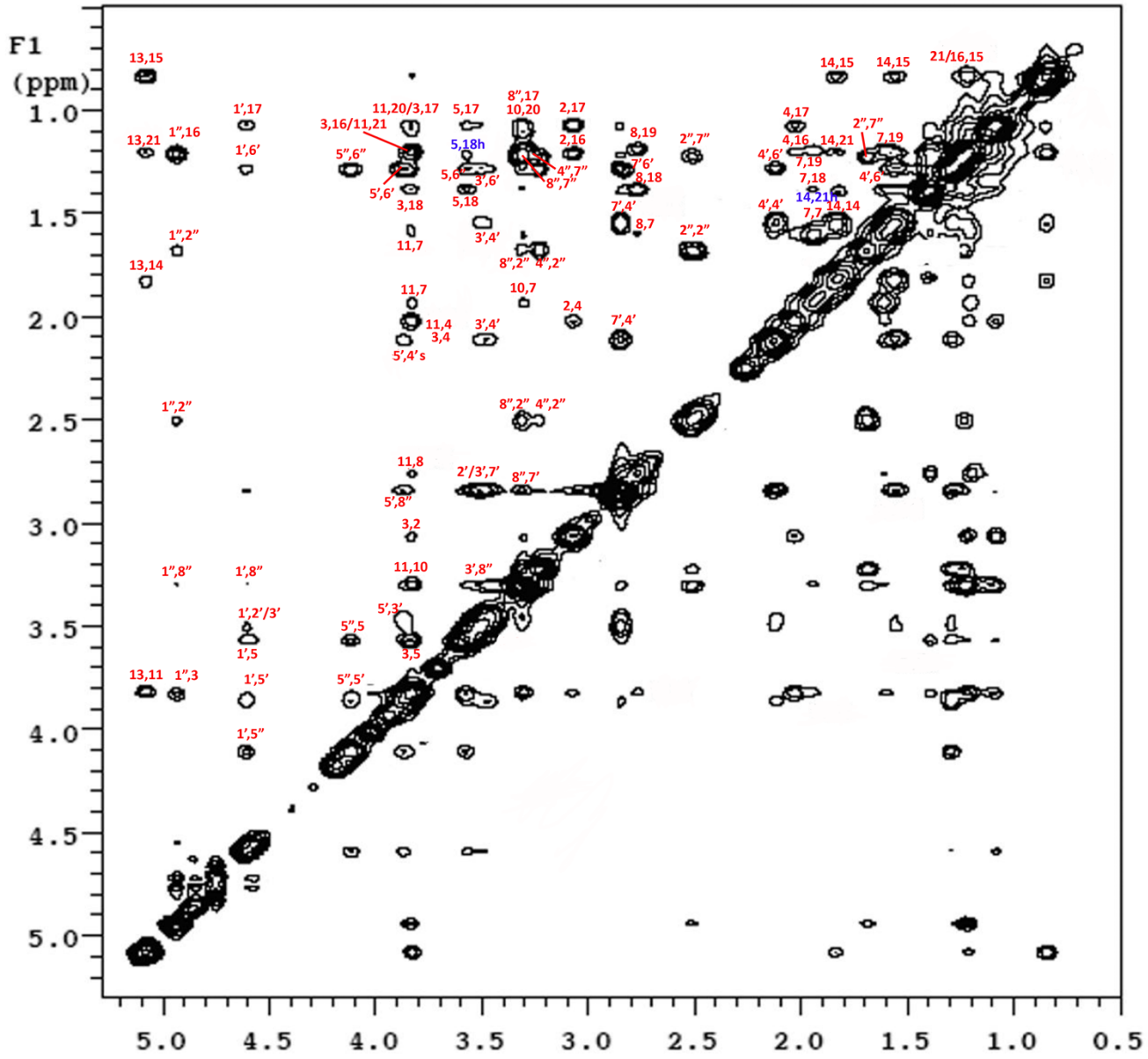

Fig S1B: 600 MHz TRNOESY NMR spectra of erythromycin A (4 mmol dm^{-3}) in the presence of 0.8 µmol dm^{-3} ribosomes in 50 mmol dm^{-3} sodium phosphate buffer in D_2O pD 7.6 (uncorrected value), mixing time 75 ms. Small signals belonging to the hemiacetal form of the drug are labelled in blue.

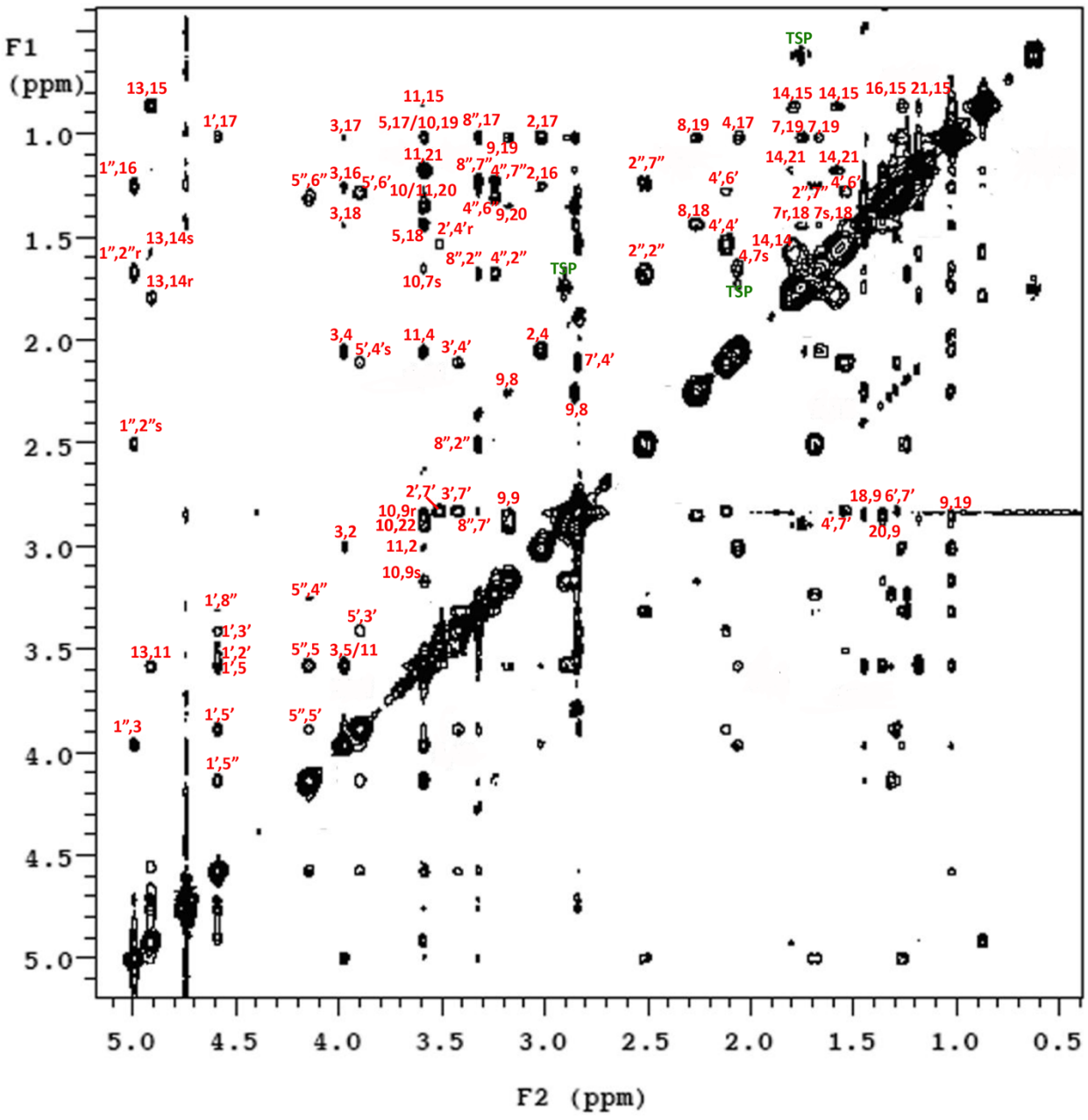

Fig S1C: 600 MHz TRNOESY NMR spectra of azithromycin (4 mmol dm^{-3}) in the presence of 0.8 μmol dm^{-3} ribosomes in 50 mmol dm^{-3} sodium phosphate buffer in D_2O pD 7.6 (uncorrected value), mixing time 75 ms.

	2	**3**	4	5	7s	7r	8	10	11	13	14r	14s	15	16	17	18	19	20	21	22	1'	2'	3'	4' r	4' s	5'	6'	7' 8'	1"	2"r	2"s	4"	5"	6"	7"	8"
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6'				m																●	s	#	s	m	m	l	●	m							
7' 8'																				●	#	l	l	m	m		l	●								s
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2"r																				●									s	●	l	m			m	m
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6"																				●	#												l	●		
7"																				●										m	m	l			●	l
8"															m					●	s		s			s		s	s	m	s				l	●
	2	**3**	4	5	7s	7r	8	10	11	13	14r	14s	15	16	17	18	19	20	21	22	1'	2'	3'	4' r	4' s	5'	6'	7' 8'	1"	2"r	2"s	4"	5"	6"	7"	8"

Table S1: 600 MHz TRNOESY connectivities for clarithromycin (4mM, top right) and erythromycin 9-ketone (bottom right) in the presence of 0.8 μM ribosomes in 50 mM sodium phosphate buffer in D_2O pD 7.6 (uncorrected value), mixing time 75 ms. Red asterisks denote signals absent in one spectrum but present in the other. Green hashes represent additional signals seen by Bertho *et al.*[1]

	2	**3**	4	5	7s	7r	8	9s	9r	10	11	13	14r	14s	15	16	17	18	19	20	21	22	1'	2'	3'	4' r	4' s	5'	6'	7' 8'	1"	2"r	2"s	4"	5"	6"	7"	8"
2	●	s	s					●	●		*					s	l																					
3	s	●	m	*				●	●		*					s	*	*				m								m								
4	l	m	●		*			●	●		l					s	m																					
5		m		●		s		●	●								*	m					m						*					l			
7s			m		●	l	s	●	●	m	*							*	*			s															
7r				*		●	s	●	●		*							s	s																		
8					*	*	●	●	●		*							l	s																		
9s							s	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
9r							m	l	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
10					s			m	m	●	m								*	m																	
11	s	m	m							*	●	m			*					m	M	m															
13											m	●	m	s	m						*																
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19					m	m	m			m									●			m															
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1"		m														m															●	m	m				
2"r																															m	●	l	m			m	m
2"s																															s	l	●	*			l	*
4"																																m		●			l	
5"				m																			m				s							s	●	m		
6"																																		m	m	●		
7"																																s	m	m			●	l
8"																	m						s							s		s	m				m	●
	2	**3**	4	5	7s	7r	8	9s	9r	10	11	13	14r	14s	15	16	17	18	19	20	21	22	1'	2'	3'	4' r	4' s	5'	6'	7' 8'	1"	2"r	2"s	4"	5"	6"	7"	8"

Table S2: 600 MHz TRNOESY connectivities for clarithromycin (4mM, top right) and azithromycin (bottom right) in the presence of 0.8 μM ribosomes in 50 mM sodium phosphate buffer in D_2O pD 7.6 (uncorrected value), mixing time 75 ms. Red asterisks denote signals absent in one spectrum, present in the other. Note that Me22 for the two drugs are not analogous.

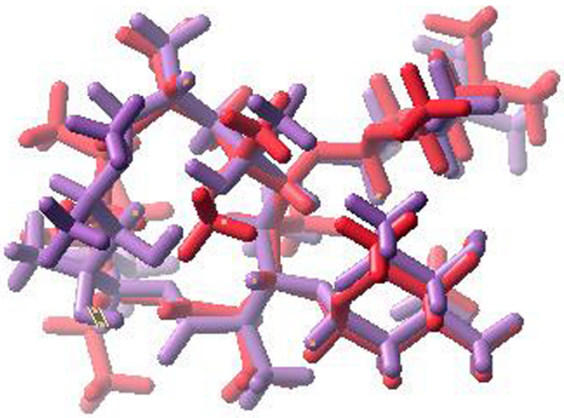

Figure S2. Superimposed folded-out structures of clarithromycin (red) and azithromycin (violet)

	Internuclear distance (Å)	
Contact	Global minimum (Macromodel)	Structure 1 (Sybyl7.3)
H4-H11	2.7	2.3
H15-H16	2.7	2.7
H3-H8	5.8	6.3
H3-H11	3.8	4.0
H4-H18	4.1	4.1
H5-H18	2.6	2.7
H16-H17	4.0	4.3

Table S3: Key internuclear distances for unconstrained modelling of clarithromycin: the global minimum of a Monte Carlo search using Macromodel (molecular mechanics) and the lowest energy conformation obtained by simulated annealing (molecular dynamics).

	Internuclear distance (Å)	
Contact	Global minimum (Macromodel)	Structure 1 (Sybyl7.3)
5-5”	2.3	2.3
16-1”	2.6	3.0
17-8”	4.1	4.1
1’-5”	2.3	4.3
5’-5”	2.7	4.3
1”-3	2.4	2.6

Table S4: Key internuclear distances for unconstrained modelling of erythromycin 9-ketone: the global minimum of a Monte Carlo search using Macromodel (molecular mechanics) and the lowest energy conformation obtained by simulated annealing (molecular dynamics).

	Internuclear distance (Å)	
Contact	Global minimum (Macromodel)	NMR constraint
4-11	4.0	2.5 ± 1
8-18	3.9	2.3 ± 1
9*proR*-18	4.7	2.5 ± 1
11-13	3.6	2.3 ± 0.5
15-16	4.9	2.5 ± 2
18-22	7.5	3.5 ± 2.5
9*proR*-21	4.0	2.3 ± 1
2"*proR*-8"	4.0	2.3 ± 1

Table S5: TRNOESY NMR data not satisfied by the Global minimum conformer for azithromycin found by unconstrained conformational search (molecular mechanics).

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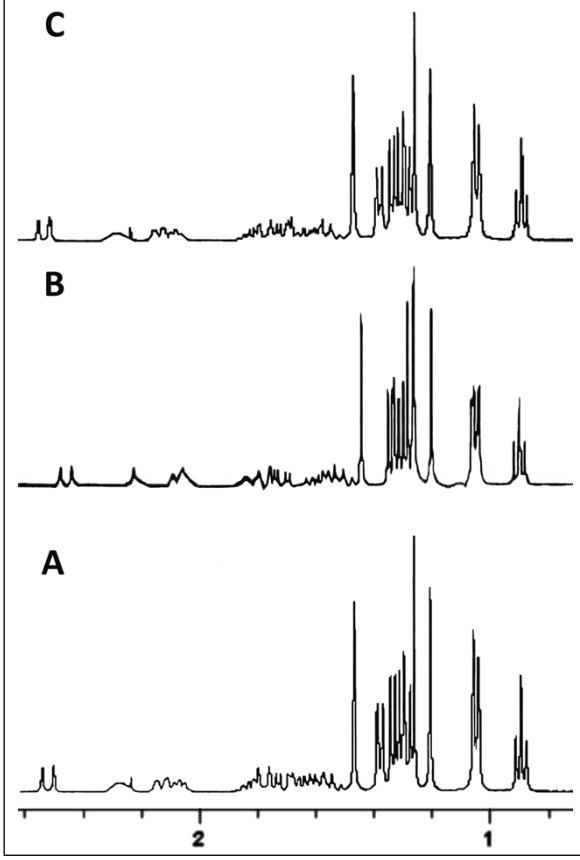

Figure S3. Part of the 500 MHz ^{1}H NMR spectra of azithromycin dissolved in buffered D_2O (uncorrected pD=7) in the temperature range 30^{0}C-90^{0}C **A**^{1}H NMR spectrum of azithromycin at room temperature; **B**^{1}H NMR spectrum of azithromycin on 90^{0}C ; **C**^{1}H NMR spectrum of azithromycin heated on 90^{0}C, and then cooled to 30^{0}C.

Assignment of the ^{1}H and ^{13}C NMR spectra of descladinosylclarithromycin and descladinosylazithromycin.

1D (^{1}H and ^{13}CDEPT45, DEPT90, DEPT135) and 2D (COSY, gHMBC and HSQC) NMR spectra of descladinosyl clarithromycin and descladinosyl azithromycin were recorded on a Bruker Avance 300 spectrometer operating at 300 MHz for ^{1}H. The spectra were run in $CDCl_3$ (^{1}H spectra were recorded also with D_2O shake). The concentration of descladinosyl clarithromycin and descladinosyl azithromycin for recording ^{1}H spectra was 4.3 mg mL^{-1} and for all other spectra 57.1 mg mL^{-1}. The ^{1}H spectra were processed using a Gaussian window function with parameters optimized empirically. The ^{13}C, DEPT45, DEPT90 and DEPT135 were processed using an EM window function with line broadening of 1.0 Hz. Two

dimensional NMR spectra were processed using a QSINE window function with other parameters optimized empirically.

The strategy for assignment of the ^{1}H and ^{13}C NMR spectra of descladinosylclarithromycin was similar to that used for other macrolide derivatives. [1]

The quaternary carbons C9 and C1 were assigned by comparison of the high frequency region of the one-dimensional ^{13}C spectrum with that of the DEPT45 spectrum. The three methylene carbons, C7, C4' and C14 were assigned non-specifically using the DEPT135 spectrum. $H_3$22 gives rise to a characteristic 3H singlet and $H_6$7'/8' to a broad 6H singlet at δ 2.95 and 2.37 respectively. $H_3$15 gives rise to a characteristic high field triplet in the 1D ^{1}H spectrum.

The wealth of information in the gHMBC spectrum (see Table S6) could now be used to walk around the molecule, assigning each carbon and hydrogen signal. Signals connecting C5 to the sugar hydrogens and H5 to the sugar carbons were used to connect the macrolide and sugar rings. Stereochemistry at C7, C14 and C4' was assigned by analogy with the spectrum of clarithromycin (chemical shifts and J-coupling information).

The strategy for assigning the NMR spectra of descladinosylazithromycin was exactly analogous.

Position	Descladinosylclarithromycin				Descladinosylazithromycin			
	Multiplicity	^{1}H (ppm)	^{13}C (ppm)	HMBC connectivities ($^{1}H\rightarrow^{13}C$)	Multiplicity	^{1}H (ppm)	^{13}C (ppm)	HMBC connectivities ($^{1}H\rightarrow^{13}C$)
1	-	-	174.0			-	176.7	
2	m	2.65	43.5	1,2,16		2.65	43.60	1
3	d	3.57	77.9	1,2,4,5,16,17		3.77	78.58	-
4	dq	2.12	34.8	3,5,17		2.28	34.96	-
5	s	3.69	87.2	4,6,7,17,18,22,1'		3.55	94.26	3, 4,6,7,17,18,1'
6	-	-	77.0	-		-	72.15	-
7	d	1.56	37.8	8,9,18,19		1.50	41.15	-
	dd p obs	1.94		6,8,9		1.60		-
8	m	2.56	44.5	9,19		1.91	25.64	-
9	**-**	**-**	219.7	-				
9	**m**					**2.54**	**69.99**	-
10	dq p obs	3.01	36.5	9,20		2.74	61.59	20
11	s	3.86	68.8	9,20,13		3.66	74.48	-
12	-	-	73.2	-		-	73.23	-
13	dd	5.18	76.3	1,11,12,15,21		4.76	76.31	1,12
14	m	1.96	20.4	15		1.85 1.45	19.91	
	m	1.50		13,15				-
15	t	0.84	9.4	13,14		0.88	9.86	13,14
16	d	1.25	14.2	1,2,3		1.30	15.01	1,2,3
17	d	1.12	7.3	3,4,5		1.12	6.82	3,4,5
18	s	1.37	17.8	5,6,7		1.32	24.87	5,6,7
19	d	1.12	16.8	8,9		0.93	20.17	7,8,9
20	d	1.13	11.6	9,10,11		1.06	15.01	10,12
21	s	1.19	15.2	11,12,13		1.08	6.86	12
22	**s**	2.98	48.6	6				
22	**s**					**2.37**	**36.10**	9,10
1′	d	4.39	105.0	5,3′		4.47	105.91	5,3′
2′	dd	3.24	75.6*	1′,3′		3.27	69.53	3′
3′	m	2.56	64.8			2..5	64.68	-
4′	dm	1.68	27.8	3′		1.25, 1.6	26.91	-
	m obs	1.28		3′				
5′	m	3.51	69.6			3.54	69.09	-
6′	d	1.26	20.2	5′,4′		1.26	20.22	4′,5′
7′/8′	s	2.27	39.2	3′		2.25	39.22	3′

Selected spectra are shown below:

[1] P. Tyson, A. Hassanzadeh, M.. N. Mordi, D. G. Allison, V. Marquez, J. Barber, MedChemComm, **2**, 331.

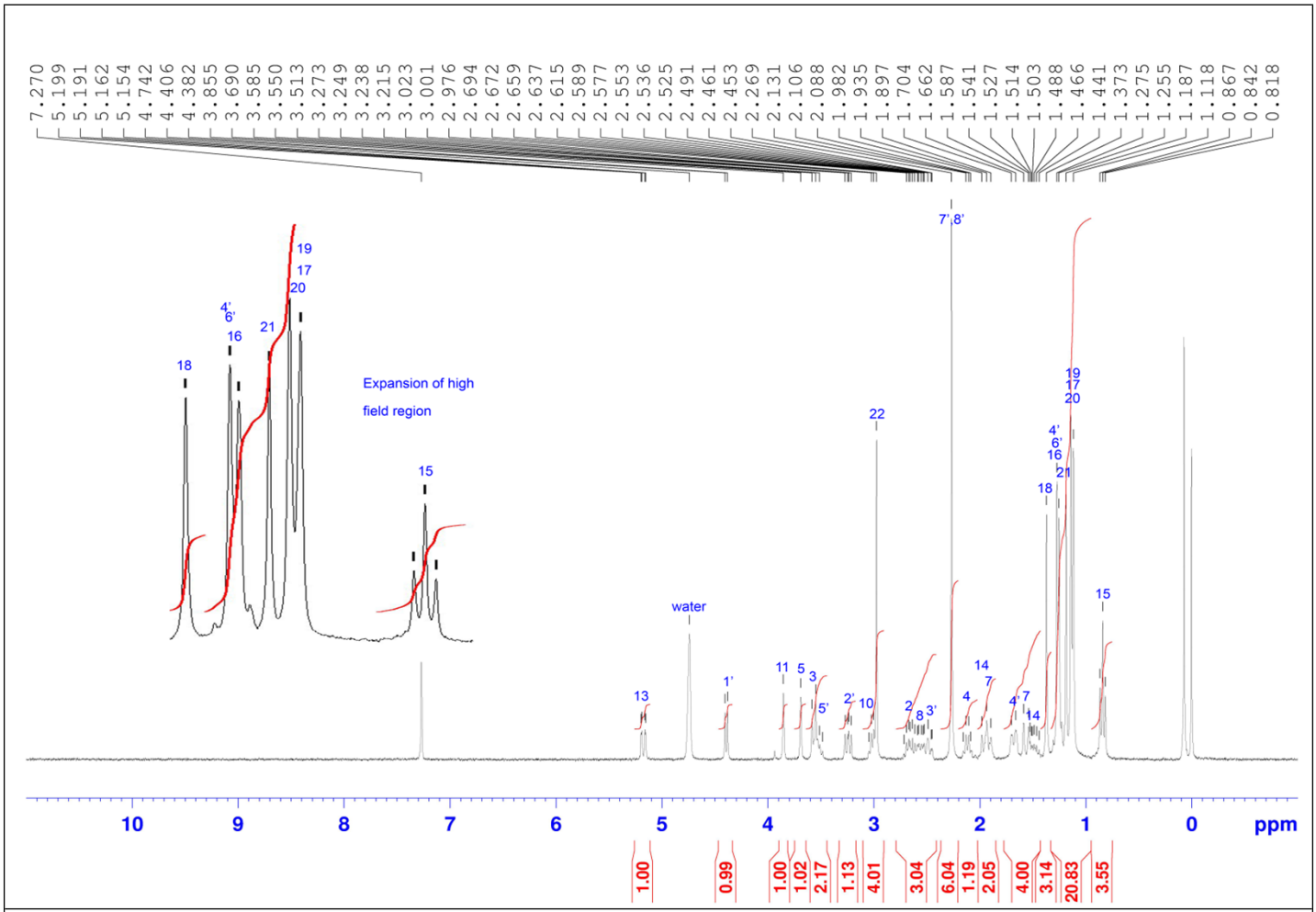

Figure S4: 300 MHz ^{1}H NMR spectrum of descladinosylclarithromycin in $CDCl_3$

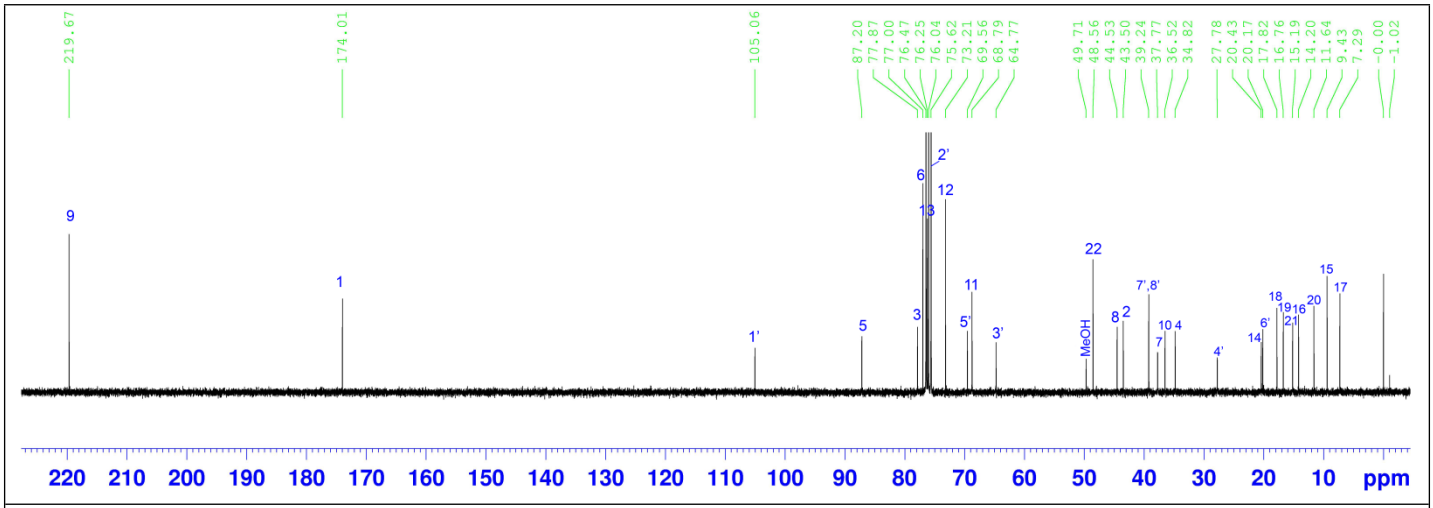

Figure S5: 300 MHz ^{13}C NMR spectrum of descladinosylclarithromycin in $CDCl_3$

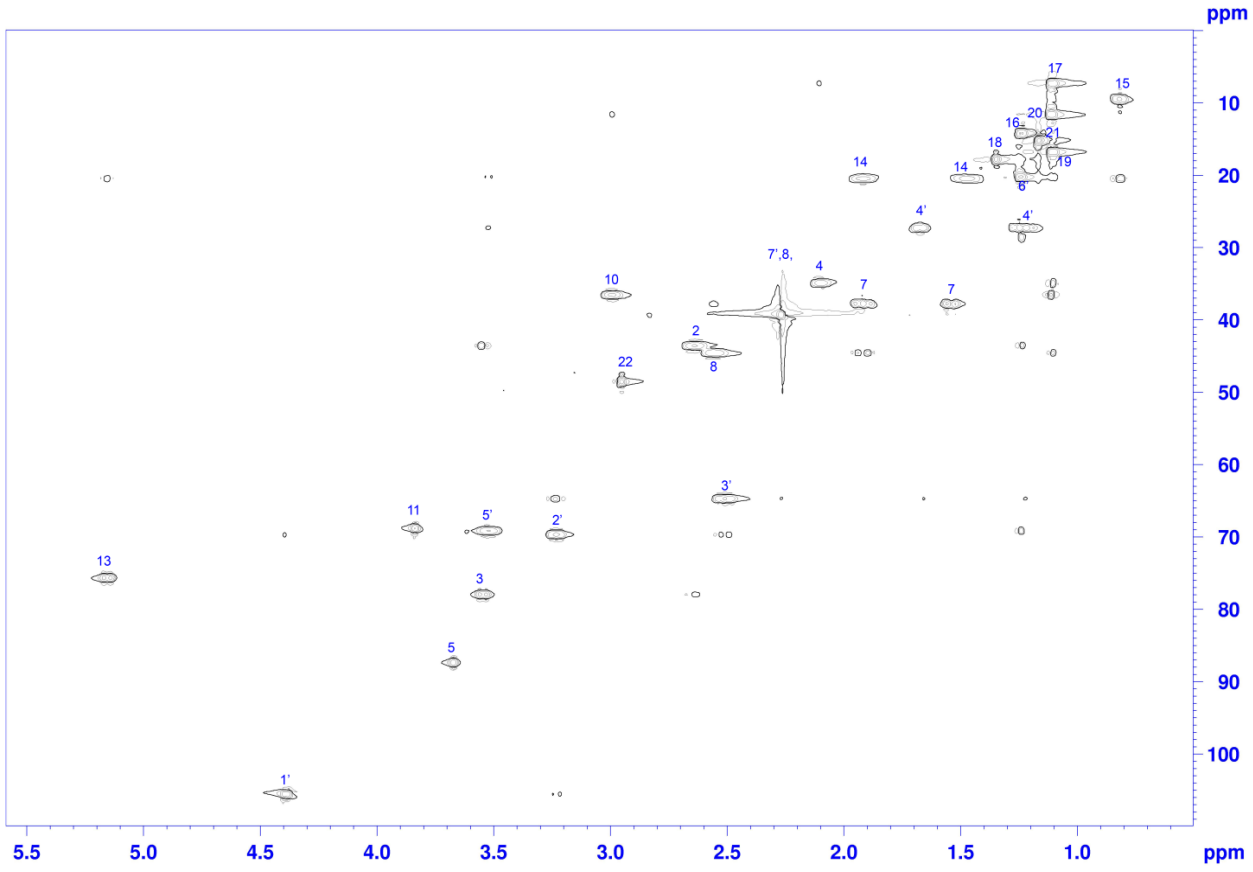

Figure S6: 400 MHz HSQC of descladinosylclarithrromycin in $CDCl_3$

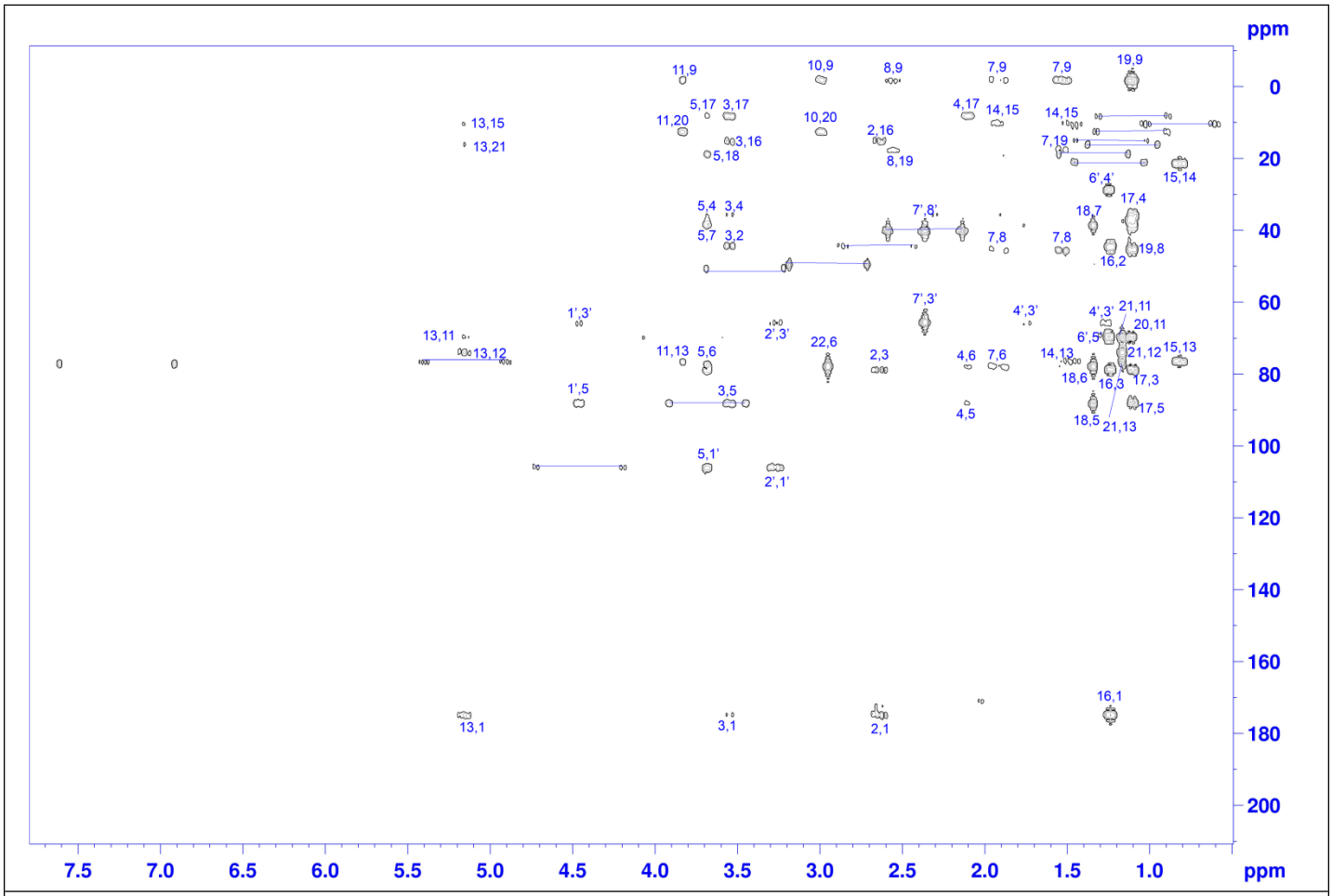

Figure S7: 300 MHz gHMBC spectrum of descladinosylclarithromycin in $CDCl_3$

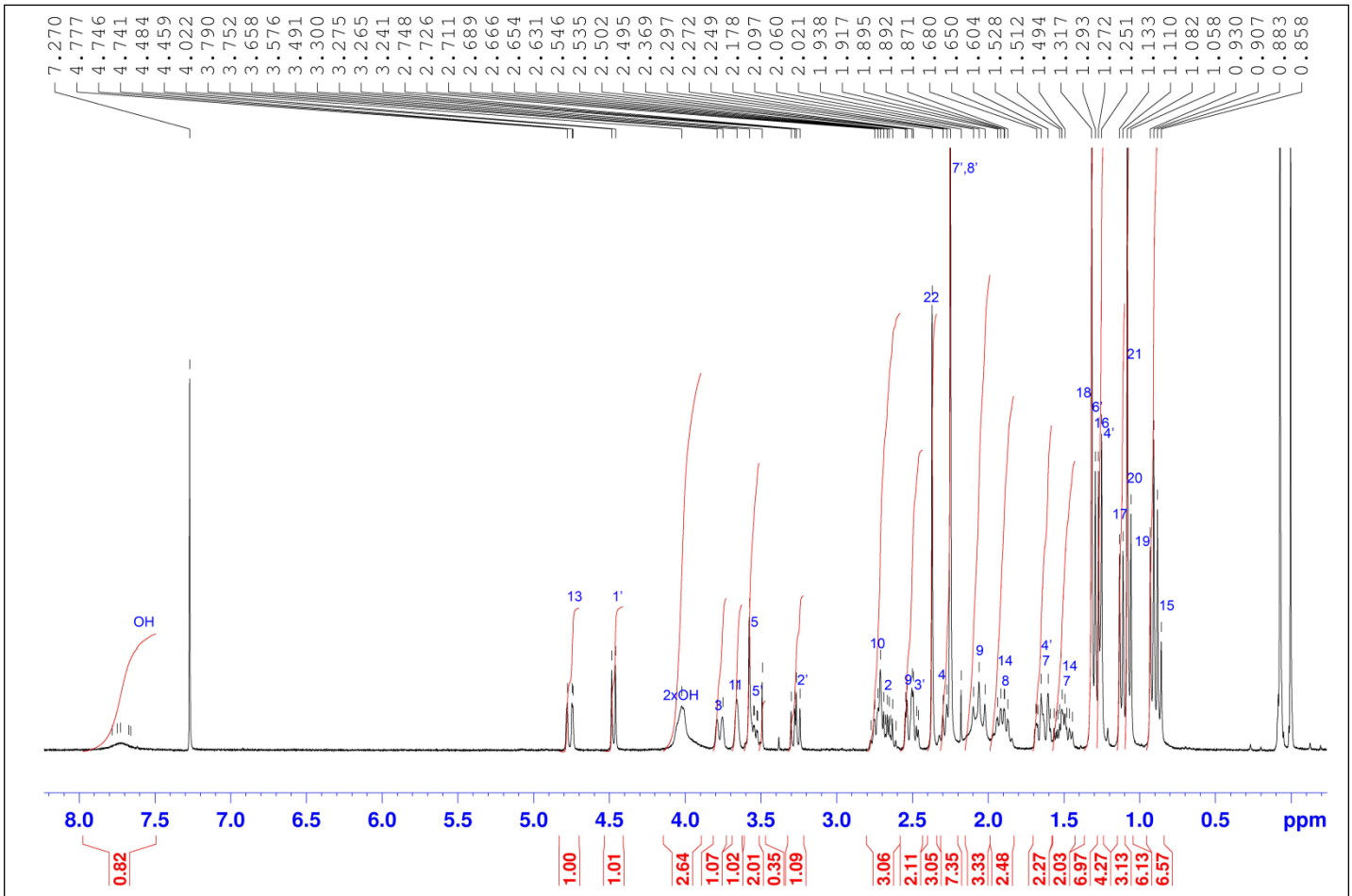

Figure S8: 400 MHz ^{1}H NMR spectrum of descladinosylazithromycin in $CDCl_3$

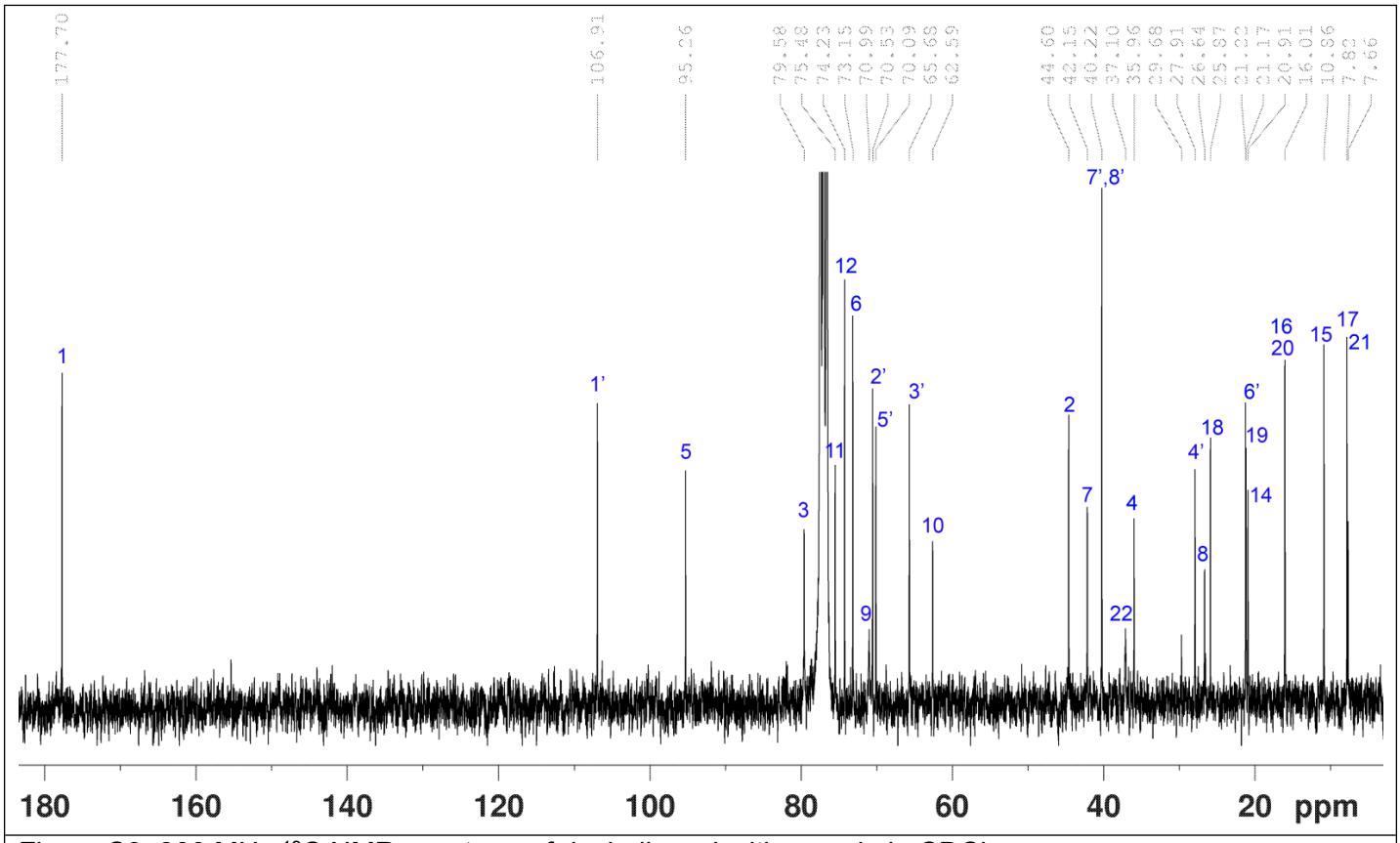

Figure S9: 300 MHz ^{13}C NMR spectrum of decladinosylazithromycin in $CDCl_3$

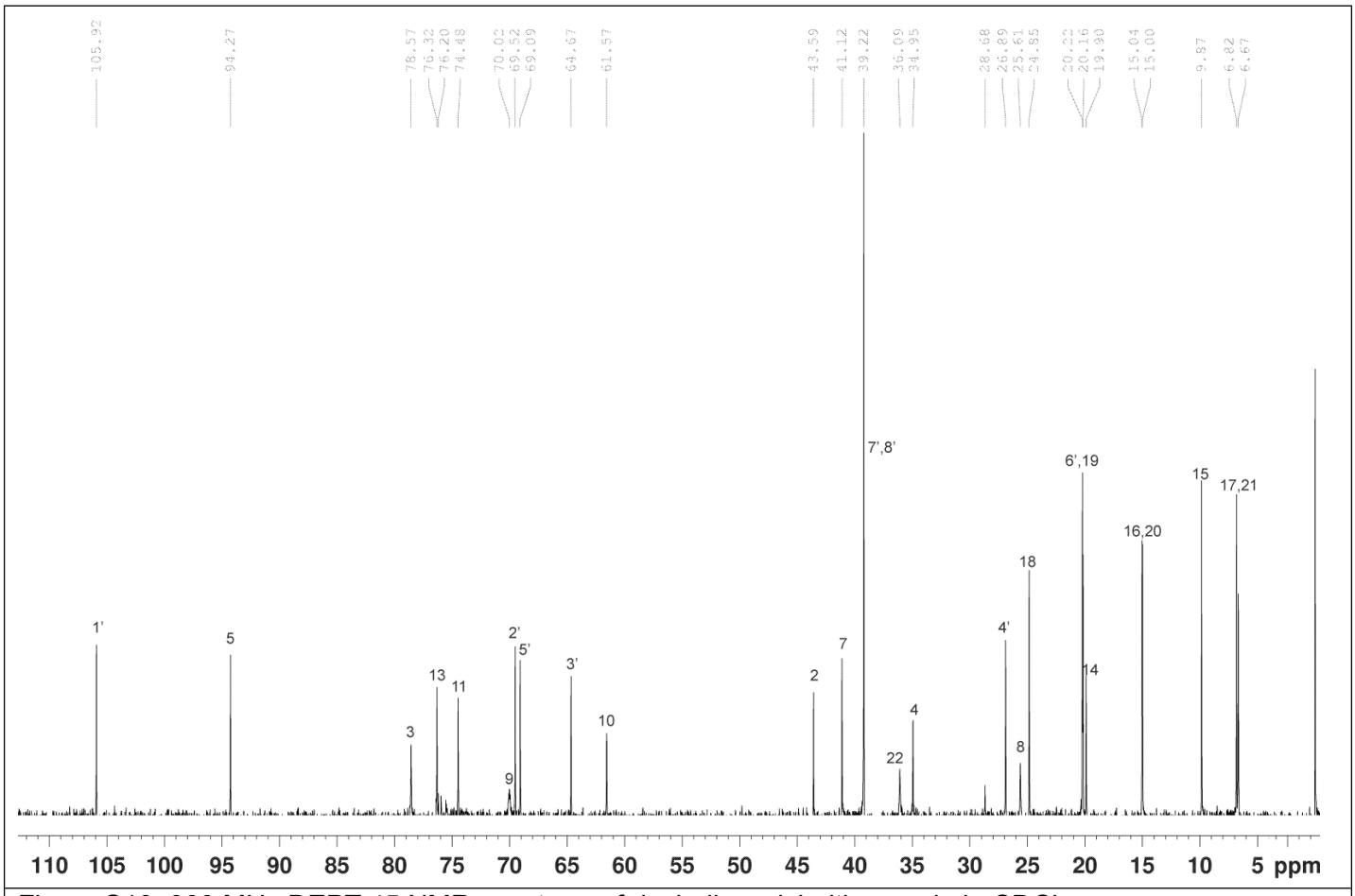

Figure S10: 300 MHz DEPT 45 NMR spectrum of decladinosylclarithromycin in $CDCl_3$

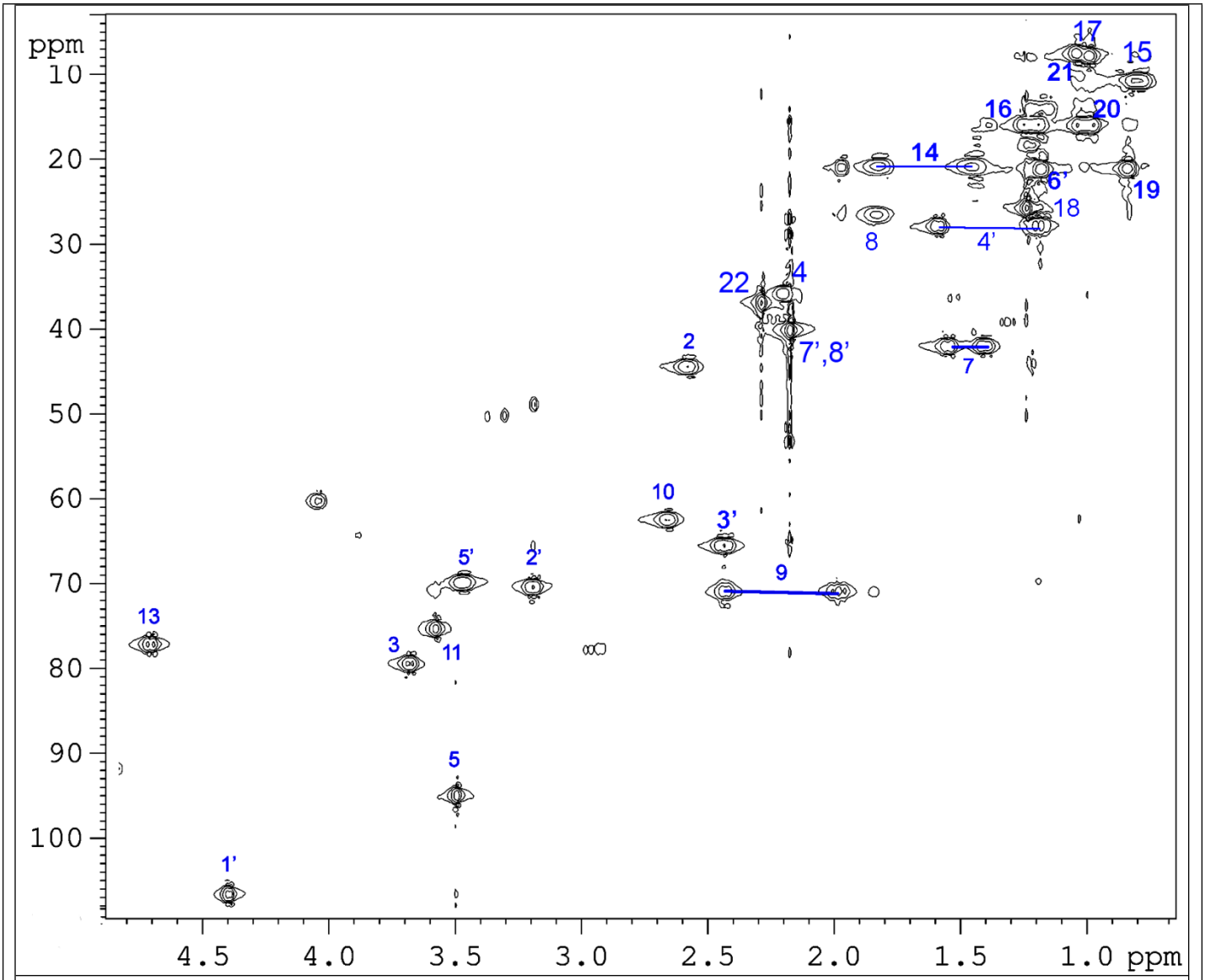

Figure S11: 500 MHz HSQC of descladinosylazithromycin in $CDCl_3$

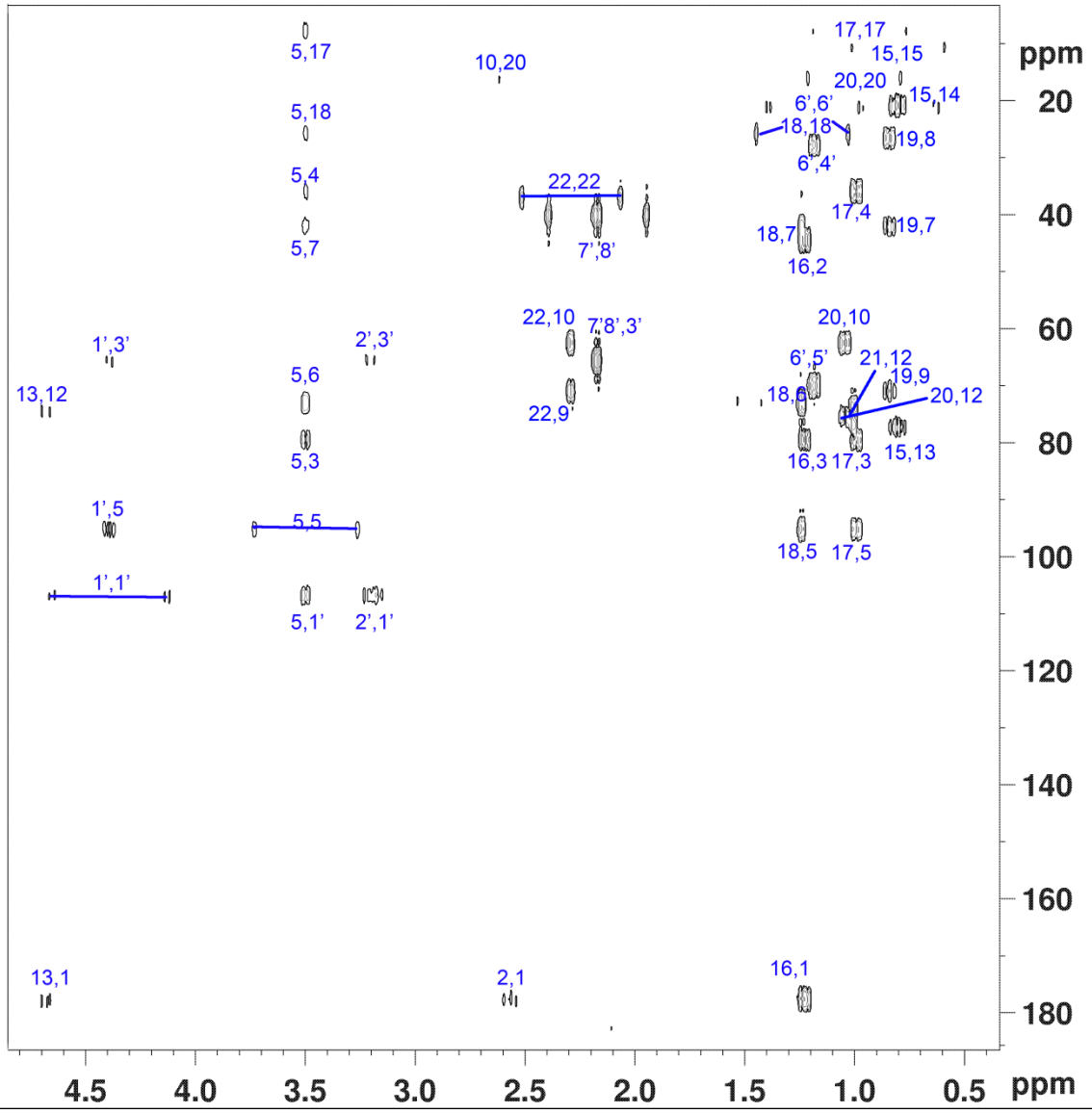

Figure S12: 300 MHz gHMBC spectrum of descladinosylazithromycin in $CDCl_3$

Organism (Gram +ve or Gram –ve)	MIC (μg mL^{-1})			
	Clarithromycin	Azithromycin	Descladinosyl clarithromycin	Descladinosyl azithromycin
Escherichia coli (-ve)	12	2	NS[1]	NS[2]
Bacillus cereus (+ve)	0.2	4	NS[2]	NS[2]
Staphylococcus aureus (+ve)	0.2	8	NS[2]	NS[2]
Pseudomonas aeruginosa (-ve)	62.5	15.6	NS[1]	NS[1]
Staphylococcus epidermidis(+ve)	250	31.2	NS[1]	NS[1]
Serratia marcescens (-ve)	NS[1]	NS[1]	NS[1]	NS[1]
Corynebacterium xerosis (+ve)	NS[1]	250	NS[1]	NS[1]

Table S7: Minimum inhibitory concentrations of clarithromycin, azithromycin and their decladinosyl derivatives against selected Gram positive and Gram negative bacteria NS[1]: MIC>1000 μg mL^{-1}; NS[2]: MIC 500-1000 μg mL^{-1}.

Experimental Procedures

Synthesis of 3-O-descladinosyl-6-O-methyl-erythromycin A

Clarithromycin (0.1 g) was dissolved in acetone (10 mL) and treated with 0.25 M HCl (2 mL) overnight. The reaction mixture was then washed with dichloromethane (3 x 15 mL) to remove free cladinose. The aqueous layer was adjusted to pH 8.5-9 by addition of saturated sodium bicarbonate solution and extracted using ethyl acetate (4 x 15 mL). The combined ethyl acetate layers were washed once with water (50 mL) and dried over anhydrous potassium carbonate. After removal of the drying agent, the solvent was evaporated to dryness to give colourless crystals. The crude product was purified using silica column chromatography (chloroform: methanol, 5:1). Yield: 31.6 mg (40%). Mp. 115 ^{0}C. $C_{30}H_{56}NO_{10}$ requires: 590.3904; found: 590.3889. The structure was confirmed using one-dimensional ^{1}H and ^{13}C and two-dimensional COSY and HMBC NMR analysis. The proton and carbon chemical shifts, multiplicities and HMBC connectivities for the 3-*O*-descladinosyl-6-*O*-methyl-erythromycin A are summarized in Table S6.

Synthesis of 3-O-descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A.

Azithromycin (200 mg) was treated with 0.25 M HCl (2 mL) overnight. The reaction mixture was washed using dichloromethane (3x15 mL). The pH of aqueous layer was adjusted to 9 using saturated solution of sodium bicarbonate and extracted using ethyl acetate (4x15 ml). The combined organic layers were washed with water (50 mL) and dried over anhydrous potassium carbonate. After removal of the drying agent, the solvent was evaporated to dryness to give colourless crystals. The crude product was purified using silica column chromatography (chloroform: methanol, 5:1).Yield 91.4 mg, 58%. Mp. 106-108^{0}C. $C_{30}H_{59}N_2O_9$ requires: 591.4221; found: 591.4213. The structure was confirmedusing one-dimensional ^{1}H and ^{13}C and two-dimensional COSY and HMBC NMR analysis. The proton and carbon chemical shifts, multiplicities and HMBC connectivities for 3-*O*-descladinosyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A are summarized in Table S7.

NMR analysis

1D (^{1}H and ^{13}C DEPT45, DEPT90, DEPT135) and 2D (COSY, gHMBC and HSQC) NMR spectra of descladinosyl clarithromycin and descladinosyl azithromycin were recorded on a Bruker Avance 300 spectrometer operating at 300 MHz for ^{1}H. The ^{1}H spectra were processed using Gaussian window function with parameters optimized empirically. The ^{13}C, DEPT45, DEPT90 and DEPT135 were processed using EM window function with LB value equals to 1.0 Hz. Two dimensional NMR spectra were processed using QSINE window function with other parameters optimized empirically (e.g. SSB was equal to 2).

We used Bruker Avance 500 spectometer operating at 500 MHz for ^{1}H for VT experiments of azithromycin.

Deuteriated *E.coli* ribosomes were prepared as previously described [2] (deuteriation reduces the possibility of spin diffusion in transferred NOE experiments). 600 MHz Spectra were run of each drug alone, in the presence of 0.8 pmol dm^{-3} ribosomes (a concentration known to give atwofold increase in linewidth of erythromycin A). The buffer in each case was 50 mmol dm^{-3} sodium phosphate in D_2O,apparent pH 7.6. The spectra were acquired at 600 MHzusing a data matrix of 2048 x 1024 points and processed with zero filling in FI.The mixing times were optimised empirically and were 75 ms.

Determination of minimum inhibitory concentrations

Inocula for broth dilution endpoint determination of bacterial antimicrobial susceptibility were prepared as follows: single colonies of anaerobic test bacteria from uncontaminated agar plates were inoculated into sterile, nutrient broth (10 mL) contained in 25 mL sterile plastic universals and incubated in a standard aerobic incubator at 37 °C for 24 h. Cultures were then diluted to concentration of 10^5 cfu/mL using MacFarland standard. Stock samples of clarithromycin, descladinosyl clarithromycin, azithromycin and descladinosyl azithromycin were prepared in distilled water. Testing was performed in 96-well microtitre plates (Becton Dickinson, Franklin Lakes, NJ, USA). Diluted overnight culture (100 µL) was delivered to each test well except the first column of the plate containing 100 µl of the antibiotic solution in distilled water diluted by 100 µl of double strength broth and the last two columns of the plate which contain 100 µl of single strength broth and 100 µl of destilled water, respectively. Doubling dilutions were then carried out across the plate using a multi-channel pipette, changing the tips at each dilution step. The plates were then incubated for 24 h in standard incubator at 37°C. The MICs were determined as the lowest concentration of antimicrobial at which growth did not occur. Growth was detected as turbidity, relative to an uninoculated well using a microtitre plate reader

[2] J. I. Gyi, R. J. Brennan, D. A.Pye and J. Barber, J. *Chem. Soc.,Chem. Commun.,* 1991, 1471.

(Anthos HTII; Anthos-Labtec Instruments, Salzburg, Austria).[3]

[3] A. J. McBain, R. G. Ledder, P. Sreenivasan, P. Gilbert, *J. Antimicrob. Chemother.*, 2004, **53**, 772.