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Structural investigation of the O-specific polysaccharides of *Morganella morganii* consisting of two higher sugars☆

Michelle Kilcoyne,[a] Alexander S. Shashkov,[b] Sof'ya A. Senchenkova,[b] Yuriy A. Knirel,[b] Evgeny V. Vinogradov,[c] Joanna Radziejewska-Lebrecht,[d] Regina Galimska-Stypa,[d] Angela V. Savage[a,*]

[a]*Department of Chemistry, National University of Ireland, Galway, Ireland*
[b]*N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow 119991, Russia*
[c]*Institute for Biological Sciences, National Research Council, Ottawa, Canada*
[d]*Department of Microbiology, University of Silesia, Katowice, Poland*

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Dedicated to Professor Horton on the occasion of his 70th birthday

Abstract

The lipopolysaccharide of the bacterium *Morganella morganii* (strain KF 1676, RK 4222) yielded two polysaccharides, PS1 and PS2, when subjected to mild acid degradation followed by GPC. The polysaccharides were studied by ^{1}H and ^{13}C NMR spectroscopy, including two-dimensional COSY, TOCSY, NOESY, $^{1}H,^{13}C$ HMQC, and HMBC experiments. Each polysaccharide was found to contain a disaccharide repeating unit consisting of two higher sugars, 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulosonic acid (a derivative of 8-epilegionaminic acid, 8eLeg5Am7Ac) and 2-acetamido-4-*C*-(3′-carboxamide-2′,2′-dihydroxypropyl)-2,6-dideoxy-D-galactose (shewanellose, She). The two polysaccharides differ only in the ring size of shewanellose and have the following structures:

PS1 →8)-α-8eLeg*p*5Am7Ac-(2→3)-β-She*f*-(1→
PS2 →8)-α-8eLeg*p*5Am7Ac-(2→3)-β-She*p*-(1→

Shewanellose has been previously identified in a phenol-soluble polysaccharide from *Shewanella putrefaciens* A6, which shows a close structural similarity to PS2.

Keywords: *Morganella morganii*; Bacterial polysaccharide structure; Lipopolysaccharide; Branched monosaccharide; 5,7-Diamino-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulosonic acid; Shewanellose; 8-epilegionaminic acid

1. Introduction

The Gram-negative bacterium *Morganella morganii* is a facultatively anaerobic prokaryote found to occur in the faeces and intestines of humans, dogs, other mammals and reptiles. It is an opportunistic secondary pathogen in patients with bacteremias,[1] respiratory tract,[2] wound[3] and especially urinary tract infections,[4,5] particularly with those of nosocomial origin.[6]

M. morganii was formerly classified as a member of the genus *Proteus*. However, its DNA was found to contain 50 mol% G + C, which is more like the DNA base composition of *Escherichia coli* and *Salmonella* rather than *Proteus*. DNA/DNA hybridization studies also showed that the organisms are related at only a 20% level to most enteric bacteria and not more than 20% to other *Proteeae*. On the basis of these criteria and other biochemical, morphological and serological

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* Corresponding author. Tel.: +353-91-750447; fax: +353-91-525700

E-mail address: angela.savage@nuigalway.ie (A.V. Savage).

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data, the species was moved to the new genus, *Morganella*, and is its only species.[6,7]

An interesting feature of the lipopolysaccharide core of *M. morganii* is the presence of two D-galacturonic acid residues, one in a terminal position and the other chain-linked. This is in common with other distantly related *Enterobacteriaceae*, such as *Proteus mirabilis*, *Providencia rettgeri*, and *Serratia marcescens*.[8]

In this paper we report on the structure determination of the O-specific polysaccharide chains of the lipopolysaccharide of *M. morganii*, type strain FK 1676 (RK 4222).

2. Results and discussion

The LPS was isolated from the water phase after the phenol–water extraction.[9] A portion of the LPS was subjected to acid hydrolysis with dilute acetic acid to yield, after fractionation by GPC, three fractions: a polysaccharide (PS1), a core oligosaccharide, and a low-molecular mass fraction containing 3-deoxyoct-2-ulosonic acid. A further portion of LPS was degraded under milder acidic conditions at pH 4.5 to give another polysaccharide (PS2). Acetic acid degradation of the precipitate obtained after degradation at pH 4.5 resulted in PS1.

The ^{13}C NMR spectrum of PS1 contained 23 signals including one of double intensity. The anomeric region contained four signals between δ 99.2 and 112.0. However, a DEPT experiment showed that only one of these, that at δ 112.0, belongs to a proton-bearing carbon, whereas the other three were quaternary carbons. In addition, the ^{13}C NMR spectrum showed signals for three nitrogen-bearing carbons at δ 61.4, 57.4, and 56.0, two C–CH_2–C groups at δ 42.5–50.9, two *N*-acetyl groups at δ 23.3 (2 C, Me) and δ 174.3–174.9 (CO), an *N*-acetimidoyl group (Am) at δ 20.3 (Me) and C=N at δ 168.1.[10,11] There were also two more C–CH_3 signals at δ 19.3 and 18.5, four signals in the region of oxygen-bearing carbons at δ 68.4–77.0, and another signal for a carbon that bears one or two oxygens at δ 92.4.

The ^{1}H NMR spectrum of PS1 (Fig. 1) contained, inter alia, signals for one anomeric proton at δ 5.27, two *N*-acetyl groups at δ 2.02 and 2.03 (6 H), one *N*-acetimidoyl group at δ 2.24,[10,11] two C–CH_2–C groups at δ 1.74/2.59 and δ 2.56/2.52, and two C–CH_3 groups at δ 1.32 and 1.38. An H-detected ^{1}H, ^{13}C HMQC experiment showed correlations of methylene protons at δ 1.74/2.59 to carbon at δ 42.5 and those at δ 2.56/2.52 to carbon at δ 50.9.

The ^{1}H and ^{13}C NMR spectra of PS1 were assigned using 2D COSY, TOCSY, NOESY, and ^{1}H, ^{13}C

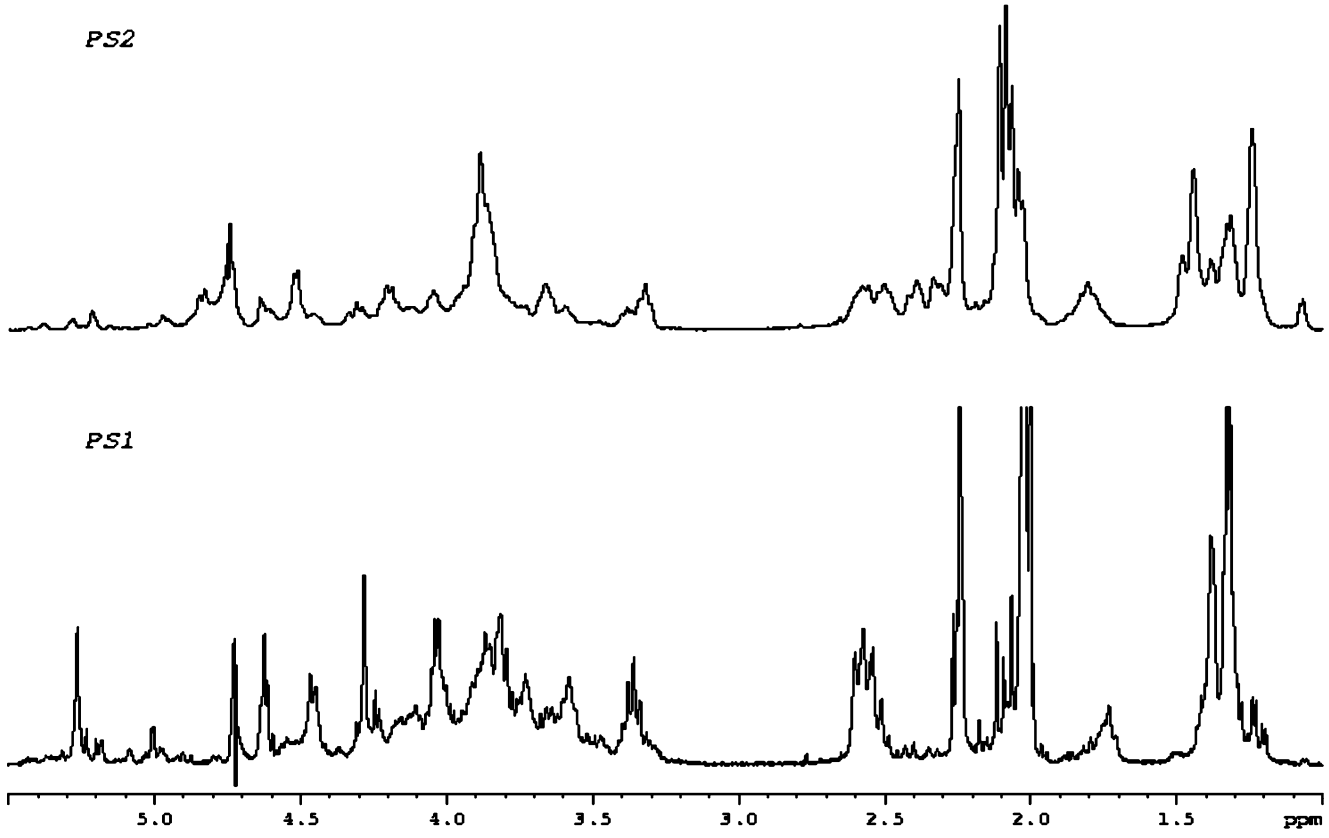


Fig. 1. 500-MHz ^{1}H NMR spectra of PS1 and PS2.

Table 1
^{1}H and ^{13}C NMR data of PS1 and PS2 (δ, ppm; J, Hz)

Sugar residue	H-1 C-1	H-2 C-2	H-3ax C-3	H-3eq	H-4 C-4	H-5 C-5	H-6 C-6	H-7a C-7	H-8 (H-7b) C-8	H-9 C-9	NAc	NAm
PS1												
→8)-α-8eLeg*p*5Am7Ac-(2→			1.74 $J_{3,3}$ 13.5	2.59 $J_{3eq,4}$ 4	3.59 $J_{3ax,4}$ 12.5	3.36 $J_{4,5}$ 10	4.45 $J_{5,6}$ 10	3.80 $J_{6,7}$ <2	3.87 $J_{7,8}$ 10	1.38 $J_{8,9}$ 6	2.03	2.24
	175.0	99.4	42.5		68.4	57.4	73.4	56.0	77.0	19.3	174.9 23.3	168.1 20.3
→3)-β-She*f*-(1→	5.27 $J_{1,2}$ <2	4.28 $J_{2,3}$ <2	4.62			4.03 $J_{5,6}$ 6.5	1.32	2.56 $J_{7,7}$ 15	2.52		2.02	
	112.0	61.4	92.4		99.2	71.1	18.5	50.9	106.6	176.0	174.3 23.3	
PS2												
→8)-α-8eLeg*p*5Am7Ac-(2→			1.78	2.55	3.57	3.33	4.45	3.88	3.85	1.46	2.10	2.25
	175.4	99.2	42.5		68.5	57.4	74.0	55.2	79.4	19.4	175.0 23.3	168.1 20.1
→3)-β-She*p*-(1→	4.51 $J_{1,2}$ 7.3	4.20 $J_{2,3}$ 10	3.91			3.84 $J_{5,6}$ 5.8	1.24	2.50 $J_{7,7}$ 15	2.51		2.08	
	104.0	51.9	85.7		79.4	74.4	15.6	49.0	105.8	177.7	174.6 23.6	

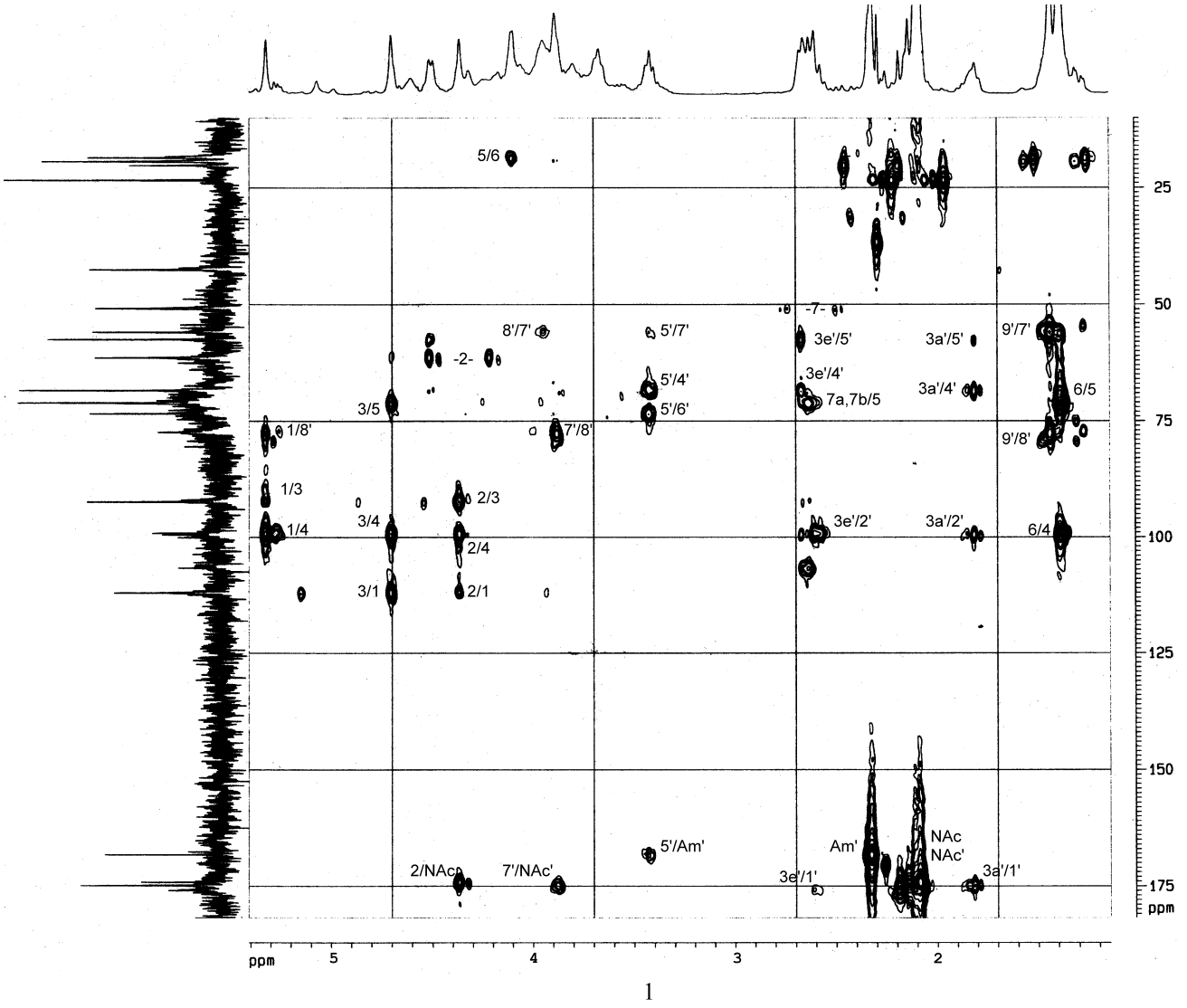


Fig. 2. 500-MHz HMBC spectrum of PS1 (8eLeg*p*5Am7Ac = X′; She*f* = X).

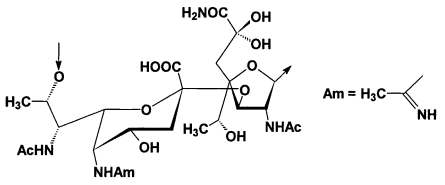
HMQC experiments (Table 1). The COSY spectrum showed three spin systems, and the NOESY spectrum revealed that two of these systems were related to one another and constituted a branched-sugar unit. On the basis of characteristic chemical shift and coupling constant values compared to published data,[10–12] the constituent sugar residues were identified as 2,6-dideoxy-2-acetamido-4-*C*-(3′-carboxamide-2′,2′-dihydroxypropyl)-D-galactofuranose (shewanellose, She*f*) and a derivative of 5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (8-epilegionaminic acid, 8eLeg*p*). Both shewanellose and 8-epilegionaminic acid have been previously found and their identification described in detail in a phenol-soluble polysaccharide from *Shewanella putrefaciens* A6.[10]

The chemical shifts of C-1 and C-2 of She*f* (δ 112.0 and 61.4, respectively) were typical of the *trans* orientation in β-galactofuranosides,[13] and, hence, shewanellose is β-linked. The anomeric configuration of the 8eLeg residue was inferred to be α based on typical chemical shifts of H-3ax and H-3eq (δ 1.74 and 2.59, respectively, compare published data[11]). A ^{1}H, ^{13}C HMBC experiment (Fig. 2) showed a correlation between C=N of the *N*-acetimidoyl group and H-5 of 8eLeg, thus indicating that this group is linked to N-5 of 8eLeg. Hence, N-7 of 8eLeg and N-2 of She are *N*-acetylated.

The chemical shift of δ 92.4 for the C-3 signal, which appeared slightly downfield compared to that in unsubstituted She*f* at δ 90.1,[10] suggested that this is the position of substitution. A low-field position at δ 77.0 of the C-8 signal of 8eLeg*p* demonstrated substitution of this monosaccharide at O-8.[11] This was confirmed by the NOESY spectrum, which showed a strong inter-residue She H-1/8eLeg H-8 peaks at δ 5.27/3.87, together with She H-1/8eLeg H-9 and She H-2/8eLeg H-9

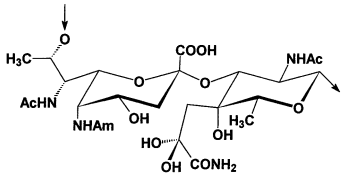
cross-peaks. The ^{1}H, ^{13}C HMBC spectrum (Fig. 2) was in agreement with these data showing She H-1/8eLeg C-8 and 8eLeg H-8/She C-1 cross-peaks at δ 5.27/77.0 and 3.87/112.0, respectively. The attachment of 8eLeg to She was demonstrated by a She H-3/8eLeg C-2 cross-peak at δ 4.62/99.4 in the HMBC spectrum.

Therefore, the following structure of the repeating unit of PS1 was established:



→8)-α-8eLeg*p*5Am7Ac-(2→3)-β-She*f*-(1→

Examination of the ^{1}H (Fig. 1) and ^{13}C NMR spectra for PS2 showed the presence of a minor series of signals identical to the spectra of PS1 and the major series with a marked similarity to those of PS1. *Vice versa*, the spectra of PS1 contained a minor series of signals identical to those of PS2. The ^{1}H and ^{13}C NMR spectra of PS2 were assigned using 2D COSY and ^{1}H, ^{13}C HMQC experiments (Table 1), and the same two monosaccharides as in PS1 were identified on the basis of characteristic coupling constant and chemical shift values.[10,11] However, while 8eLeg5Am7Ac remained in the pyranosidic form, the coupling constant values for She indicated the pyranosidic rather than the furanosidic form (Table 1) (compare published data[10]). The ^{13}C NMR chemical shift data of PS2 demonstrated the same substitution pattern as in PS1 and, therefore, the structure of the repeating unit of PS2 is as follows:



→8)-α-8eLeg*p*5Am7Ac-(2→3)-β-She*p*-(1→

Absolute configurations of the residues were not determined in this project but were fully established in previous work on a polymer of these sugars with pyranosidic conformations.[10] Thus, full agreement of the ^{1}H and ^{13}C spectra for one of the polymers (PS2) allowed us to propose the same absolute configurations i.e. L-*glycero*-D-*galacto* for 8eLeg and D-*galacto* for She.

In conclusion, it was found that the lipopolysaccharide from *M. morganii* includes two different O-specific polysaccharide chains having similar disaccharide repeating units of two higher sugars, 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-non-2-ulosonic acid (8eLeg5Am7Ac) and 2-acetamido-4-*C*-(3′-carboxamide-2′,2′-dihydroxypropyl)-2,6-dideoxy-D-galactose (She). Shewanellose exists in the furanosidic form in PS1, while in PS2 it occurs in the pyranosidic form.

From these two unusual higher sugars, 8-epilegionaminic acid with various *N*-acyl derivatives has been found in the O-specific polysaccharides of *Pseudomonas aeruginosa*, *Salmonella arizonae*, *Yersinia ruckerii*, and *S. putrefaciens*[12] and references cited therein. Shewanellose has been previously identified only once, in the polysaccharide of *S. putrefaciens* having the following structure.[10]

→4)-α-8eLeg*p*5Ac7Am8Ac-(2→3)-β-She*p*-(1→

The polysaccharide of *S. putrefaciens* shows a remarkable similarity to PS2 of *M. morganii*; the differences include the position of the *N*-acetyl and *N*-acetimidoyl groups in 8eLeg, the position of glycosylation of 8eLeg, and the presence of an *O*-acetyl group at position 8 of 8eLeg in the polysaccharide of *S. putrefaciens*. Interestingly, the polysaccharide of *S. putrefaciens* was phenol-soluble, whereas the shewanellose-containing lipopolysaccharide of *Morganella* was isolated from the water phase of the phenol–water extract.

3. Experimental

Growth of bacteria, isolation and degradation of the lipopolysaccharide.—The bacterium was provided by the strains collection of the Max Planck Institute in Freiburg, Germany. Cells were cultivated at 37 °C on TSB medium supplemented with 1% glucose in a fermentor. The wet bacterial mass (354 g) was subjected to the hot phenol–water extraction,[9] and the lipopolysaccharide (2.5 g) was isolated from the water phase.

The lipopolysaccharide (800 mg) was hydrolysed with 1% HOAc at 100 °C for 1 h with a yield of a carbohydrate material and lipid A ($\sim$45% and $\sim$30% of the lipopolysaccharide weight, respectively). The former was separated by centrifugation and fractionated by GPC on a Sephadex G-50 (F) column in 0.05 M pyridinium acetate buffer, pH 5.4, to give three fractions, including a polysaccharide (PS1, 104.2 mg), a core oligosaccharide (105.0 mg), and a 3-deoxyoct-2-ulosonic acid-containing fraction (42.6 mg).

Another portion of the lipopolysaccharide (92.4 mg) was subjected to hydrolysis with 0.1 M NaOAc–HOAc buffer, pH 4.5, at 100 °C for 2 h, the precipitate (59.9 mg) was removed by centrifigation and the water-soluble material was fractionated on a Sephadex G-50 column to yield an undegraded lipopolysaccharide (5.6 mg) and a polysaccharide (PS2, 7.3 mg). The precipitate was heated with 2% HOAc at 100 °C for 2 h, and PS1 (8.5 mg) was isolated from the water-soluble fraction by GPC on Sephadex G-50.

NMR spectroscopy.—Samples were deuterium-exchanged by freeze-drying three times from D_2O and then examined in solutions of 99.96% D_2O, using internal acetone as reference (δ_H 2.225, δ_C 31.45). NMR spectra were recorded at 30 °C on a JEOL Lambda 400 MHz spectrometer equipped with a DEC AXP 300 computer workstation or a Bruker DRX-500 MHz spectrometer and processed using standard Bruker software (XWINNMR 1.2). The mixing time in the NOESY experiment was 500 ms. In the TOCSY experiment the duration of the MLEV17 spin-lock was 80 ms. Other 2D parameters were essentially the same as previously described.[14]

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