SWALPAMYCIN, A NEW MACROLIDE ANTIBIOTIC

II. STRUCTURE ELUCIDATION

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A new antibiotic swalpamycin (**1**) has been isolated from the culture broth of *Streptomyces* sp. Y-84,30967. The antibiotic has the molecular formula of $C_{37}H_{56}O_{14}$ and belongs to the class of 16-membered neutral macrolide antibiotics. Its structure has been elucidated by an analysis of its spectral properties. It contains a novel aglycone herein called swalpanolide.

In the preceding paper[1] we have described the isolation, purification and the biological properties of swalpamycin (**1**), a novel macrolide antibiotic. This account deals with the structure elucidation of this new compound. Table 1 summarizes the physico-chemical properties of swalpamycin.

The high resolution electron impact mass spectrum (HREI-MS) of the trimethylsilyl derivative (**2**) of swalpamycin showed the parent peak at *m/z* 868.4470 corresponding to a molecular formula of $C_{37}H_{54}O_{14}$ $(Si(CH_3)_3)_2$. This implies a molecular formula of $C_{37}H_{56}O_{14}$ for swalpamycin and indicates the presence of two OH groups and an unsaturation number of 10. The UV absorption maxima at 216 and 280 nm suggest the presence of an α,β-unsaturated lactone and an $\alpha,\beta,\gamma,\delta$-unsaturated ketone respectively. The IR spectrum shows bands due to hydroxy (3440 cm^{-1}), α,β-unsaturated lactone (1710, 1650 cm^{-1}), $\alpha,\beta,\gamma,\delta$-unsaturated ketone (1680, 1630, 1595 cm^{-1}) groups; and a strong band at 1800 cm^{-1} which is characteristic of a cyclic carbonate moiety. The ^1H NMR spectrum (Fig. 1 and Table 2) of swalpamycin in $CDCl_3$ at 400 MHz shows seven CH_3 doublets in the region of δ 0.95~2.0, two OCH_3 singlets; and a pair of doublets ($J \cong 8$ Hz) centered at δ 4.58 and 4.60 corresponding to two anomeric protons. The corresponding carbon signals appear in the ^{13}C NMR spectrum (Table 3), both the anomeric carbons resonating at δ 101.1.

Swalpamycin has therefore two sugar units, and from the large J values of the anomeric protons and the relatively low field chemical shift of the anomeric carbons it can be inferred that

Table 1. Physico-chemical properties of swalpamycin.

Appearance	White amorphous solid
MP (°C)	126~129
$[\alpha]_D^{20}$	−3.8° (*c* 2.1, $CHCl_3$)
UV λ_{max}^{MeOH} nm (log ε)	216 (5.79), 280 (5.84)
MW (EI-MS) *m/z*	724
Solubility	Soluble in MeOH, acetone, $CHCl_3$, EtOAc Insoluble in hexane, H_2O
Rf* $CHCl_3$ - MeOH (93 : 7)	0.51
EtOAc	0.48

* TLC silica gel: Merck 5554.

Fig. 1. ^{1}H NMR spectrum of swalpamycin (**1**) at 400 MHz in $CDCl_3$ (TMS internal standard).

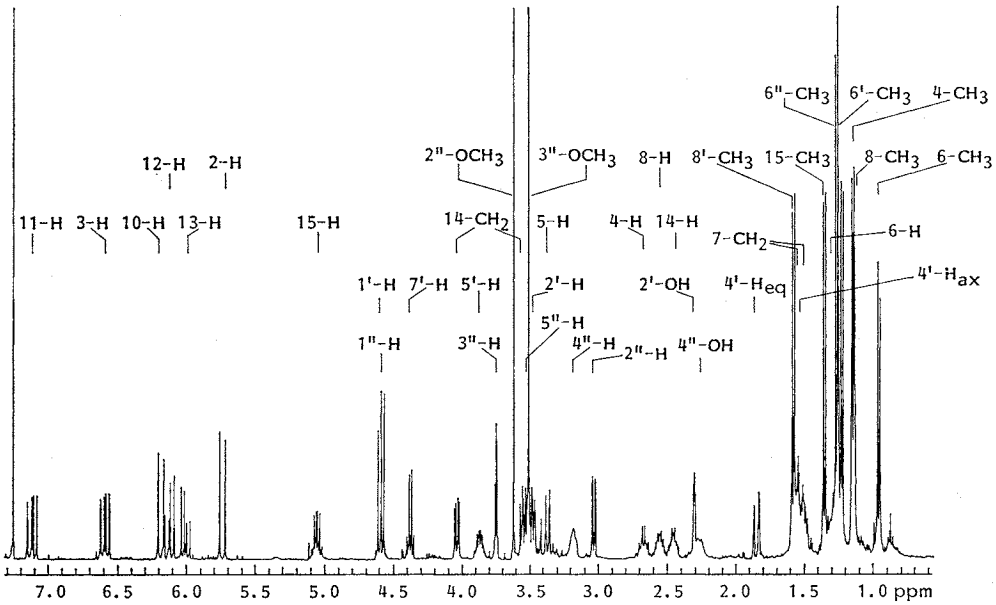


Table 2. ^{1}H NMR chemical shifts (ppm) of swalpamycin[a].

Chemical shift	Multiplicity	Coupling constant (Hz)	Assignment	Chemical shift	Multiplicity	Coupling constant (Hz)	Assignment
7.11	dd	10.9, 14.9	11-H	4.60	d	7.7	1′-H
6.59	dd	10.0, 15.5	3-H	4.38	q	6.5	7′-H
6.18	d	14.9	10-H	3.88	dqd	11.0, 6.2, 2.1	5′-H
6.11	dd	10.9, 15.4	12-H				
6.02	dd	9.2, 15.4	13-H	3.47	dd	7.7, 2.1	2′-H
5.74	d	15.5	2-H	1.85	dd	14.2, 2.1	4′-H_{eq}
5.05	dq	10.0, 6.4	15-H	1.58	d	6.5	8′-H_3
4.04	dd	3.8, 9.6	14-CH_2(1)	1.54	m	—	4′-H_{ax}
3.55	m	—	14-CH_2(2)	1.24	d	6.2	6′-H_3
3.37	d	10.1	5-H	4.58	d	7.9	1″-H
2.67	m	—	4-H	3.75	dd	2.8, 3.2	3″-H
2.56	m	—	8-H	3.54	m	—	5″-H
2.46	m	—	14-H	3.18	dd	9.4, 3.2	4″-H
1.50	m	—	7-CH_2	3.04	dd	7.9, 2.8	2″-H
1.36	d	6.4	15-CH_3	1.28	d	6.2	6″-H_3
1.31	m	—	6-H	3.62	s	—	2″-OCH_3
1.17	d	6.9	4-CH_3	3.51	s	—	3″-OCH_3
1.16	d	6.8	8-CH_3	2.24~2.32	m	—	2′-OH, 4″-OH
0.97	d	6.0	6-CH_3				

[a] Spectrum recorded at 400 MHz in $CDCl_3$ using TMS as internal standard.

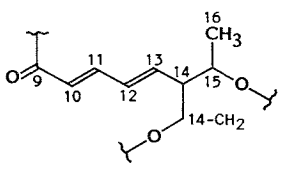
both the glycosidic linkages are of β-orientation. The ^{13}C NMR spectrum reveals the presence of three carbonyl signals at δ 203.3, 165.5 and 153.7 corresponding to the conjugated dienone, lactone and carbonate carbonyls respectively, in addition to six olefinic carbons at δ 150.9, 142.1, 141.6, 133.1, 123.4 and 121.1 all of which appear as doublets in off resonance decoupling, suggesting that the three double bonds in the molecule are disubstituted ones.

Table 3. ^{13}C NMR chemical shifts (ppm) of swalpamycin[a].

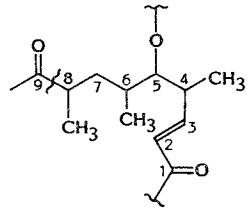
Chemical shift	Reference[b,c]	Multiplicity	Assignment	Chemical shift	Reference[b,c]	Multiplicity	Assignment
165.5	166.1	s	C-1	68.8	68.6	t	14-CH_2
121.1	120.9	d	C-2	101.1	—	d	C-1′
150.9	151.6	d	C-3	71.8	—	d	C-2′
41.2	41.3	d	C-4	84.7	—	s	C-3′
87.2	87.9	d	C-5	40.6	—	t	C-4′
33.9	34.1	d	C-6	67.2	—	d	C-5′
33.0	32.6	t	C-7	20.4	—	q	C-6′
44.7	44.9	d	C-8	81.4	—	d	C-7′
203.3	203.4	s	C-9	13.6	—	q	C-8′
123.4	123.2	d	C-10	153.7	—	s	Carbonate C=O
142.1	141.7	d	C-11				
133.1	133.0	d	C-12	101.1	101.0	d	C-1″
141.6	141.3	d	C-13	82.1	81.9	d	C-2″
51.4	49.2[d]	d	C-14	80.0	79.9	d	C-3″
69.4	72.7[d]	d	C-15	72.8	72.7	d	C-4″
18.7	25.3[d]	q	C-16	70.8	70.5	d	C-5″
19.7	19.4	q	4-CH_3	17.8	17.8	q	C-6″
17.4	17.4	q	6-CH_3	59.6	59.7	q	2″-OCH_3
18.4	17.8	q	8-CH_3	61.8	61.7	q	3″-OCH_3

[a] Spectra recorded at 22.5 MHz in $CDCl_3$ using TMS as internal standard.
[b] Reference compound is mycinamicin IV, values from ref 11.
[c] Assignments of aldgarose carbons are based on those reported in ref 3.
[d] Values at C-14, C-15 and C-16 for swalpamycin are different from reference values, since mycinamicin IV exhibits a 15-ethyl group instead of the methyl group.

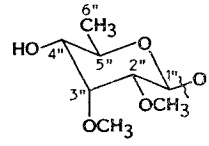
Fig. 2. Spin systems obtained from analysis of the ^1H-^1H 2D COSY spectrum of swalpamycin.



A ($C_9H_{11}O_3$)



B ($C_{11}H_{17}O_2$)

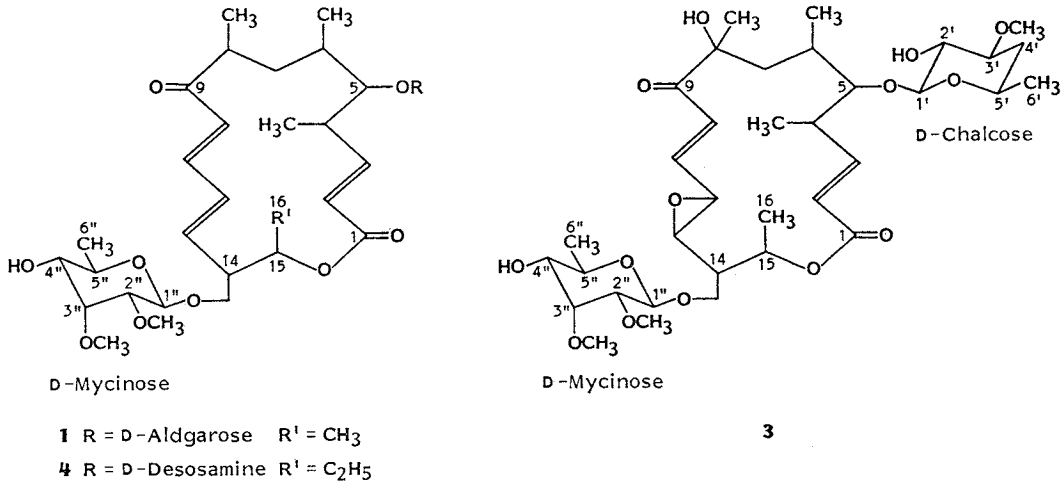


C ($C_8H_{15}O_4$)

Swalpamycin is coproduced with chalcomycin (**3**) and it is therefore reasonable to believe that the two compounds have a similar basic skeleton, *i.e.* a 16-membered lactone attached to two sugar units through glycosidic linkages. Of the known 16-membered macrolides[2], only chalcomycin, aldgamycins E, F and G[3], dedesosaminyl derivatives of mycinamicins I, IV and V are neutral compounds. A comparison of the physico-chemical and spectral properties revealed swalpamycin to be different from these antibiotics, although the basic tylosin skeleton, *i.e.* a 16-membered macrolactone having sugar attachments at C-5 and 14-CH_2, is common to all of them.

Analysis of the ^1H-^1H two-dimensional correlation spectroscopy (2D COSY) spectrum of (**1**) revealed the spin systems $C_9H_{11}O_3$ (**A**), $C_{11}H_{17}O_2$ (**B**) and $C_8H_{15}O_4$ (**C**) (Fig. 2). The coupling constants of the olefinic protons indicate that all the double bonds in swalpamycin are of *E*-configuration. The C-15 methine proton appears at δ 5.05 (dq, J=6.4 and 10.0 Hz) and its splitting pattern is identical to the 15-H of chalcomycin indicating the presence of a 15-CH_3 group (δ 1.36, d, J=6.4 Hz). The

Fig. 3. Structures of swalpamycin (1), chalcomycin (3) and mycinamicin IV (4).



aglycone unit in swalpamycin is thus different from the one reported for mycinamicin IV (4) in having a 15-methyl group instead of an ethyl group; and thereby represents a novel skeleton (Fig. 3). This fact is corroborated by the chemical shift values in the ^{13}C NMR spectrum of the aglycone part of **1** (Table 3) which are quite similar to those of **4** except for the signals due to C-14, C-15 and C-16. The geminal protons of the 14-CH_2 group appear at δ 3.55 (m) and 4.04 (dd, J=3.8 and 9.6 Hz). The signals due to 2″-H, 3″-H, 4″-H and 5″-H in the mycinose unit **C** appear at δ 3.04 (dd), 3.75 (dd), 3.18 (dd) and 3.54 (m); and the corresponding coupling constant values of $J_{1''2''}$=7.9 Hz, $J_{2''3''}$=2.8 Hz, $J_{3''4''}$=3.2 Hz and $J_{4''5''}$=9.4 Hz indicate their dispositions as axial, equatorial, axial and axial respectively. The equatorial 2″-OCH_3 singlet appears at δ 3.62 and the axial 3″-OCH_3 at δ 3.51, however when swalpamycin is acetylated with $(CH_3CO)_2O$ - pyridine, it forms a monoacetate in which the 3″-OCH_3 singlet appears at δ 3.60. Similar downfield shift of one of the mycinose OCH_3 groups is also observed† when chalcomycin is acetylated to its diacetyl derivative. Although the 2′-OH group of chalcose gets acetylated, the 3′-OCH_3 singlet (δ 3.42) is not shifted probably due to lesser perturbation in a *trans* diequatorial orientation as compared to the *cis* axial-equatorial arrangement. From all these considerations it is clear that one of the sugar units in swalpamycin is D-mycinose attached to the 14-CH_2 group of the aglycone.

The second sugar unit ($C_9H_{13}O_4$-O-) of swalpamycin has therefore to accommodate one OH, two CH_3 and a cyclic carbonate group which gives rise to the strong IR absorption at 1800 cm^{-1}. When the antibiotic is treated with base (0.1 N NaOH, 80°C, 2 hours) followed by acidification it readily loses CO_2 and the product obtained no longer shows this IR absorption. Hydrolysis with $Ba(OH)_2$ also results in immediate precipitation of $BaCO_3$ and the product does not exhibit any IR absorption at 1800 cm^{-1}. Such behaviors are typically reminiscent of the methyl aldgarosides and aldgamycins[4] which contain the sugar aldgarose having the 5-membered cyclic carbonate function. Indeed, a careful analysis of the remaining signals in the ^1H NMR spectrum of swalpamycin revealed that they correspond to the aldgarose protons[5,6]. The 7′-H appearing as a quartet (J=6.5 Hz) at δ 4.38 must be α to OCO group which, in turn, is a part of the carbonate functionality. Its coupling partner, the

† Unpublished observation from our laboratories.

Fig. 4. ^{1}H NMR (400 MHz) spectrum of swalpamycin expanded in the region where some of the aldgarose protons appear.

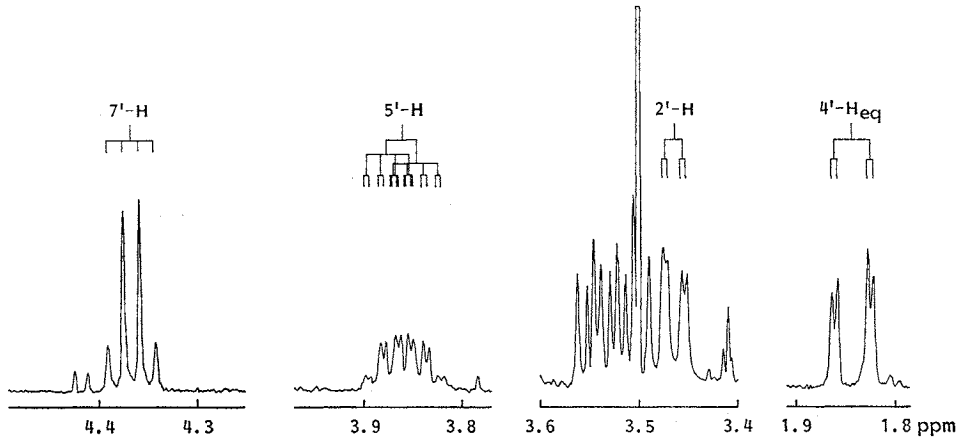
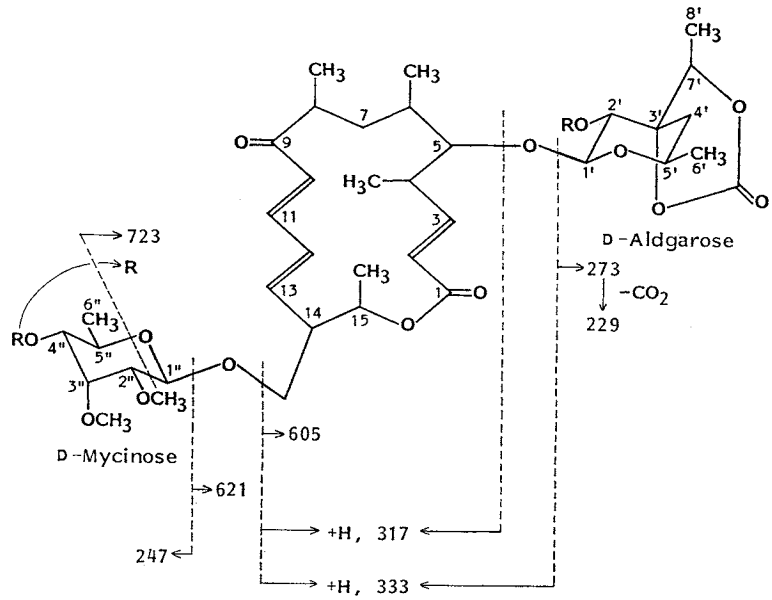


Fig. 5. Mass spectral fragmentation of trimethylsilylated swalpamycin (**2**).



1 R = H

2 R = $Si(CH_3)_3$

8'-CH_3 appears as a d (J=6.5 Hz) at δ 1.58. Absence of any other low-field resonance indicates that the other oxygen of the carbonate moiety must be attached to a quaternary carbon, *i.e.* the carbonate must be attached to a pyranose ring in a spiro fashion. The 6'-CH_3 appears at δ 1.24 (d, J= 6.2 Hz) and its coupling partner 5'-H appears at δ 3.88 (dqd, J=11.0, 6.2 and 2.1 Hz) indicating that 5'-H is attached to a carbon bearing the pyranosyl oxygen and a $>CH_{ax}CH_{eq}$ group. Indeed the 4'-H_{eq} appears at δ 1.85 as dd (J=14.2 and 2.1 Hz) and 4'-H_{ax} signal appearing at δ 1.54 is overlapped with the 7-CH_2 multiplet from the aglycone. J-resolved spectra reveal its fine structure as dd (J=

Table 4. HR-MS data of trimethylsilyl derivative of swalpamycin.

Measured mass (*m/z*)	Relative abundance	Formula	Deviation of measured mass	Ion structure
868.4470	0.07	$C_{43}H_{72}O_{14}Si_2$	+0.0010	M^+
853.4245	0.3	$C_{42}H_{69}O_{14}Si_2$	+0.0019	$M-CH_3$
723.3613	1.2	$C_{36}H_{59}O_{11}Si_2$	+0.0017	
621.3098	3.9	$C_{32}H_{49}O_{10}Si$	+0.0004	
605.3150	19	$C_{32}H_{49}O_9Si$	+0.0004	
333.2051	17	$C_{20}H_{29}O_4$	−0.0015	See Fig. 5
317.2114	18	$C_{20}H_{29}O_3$	−0.0003	
273.1158	3.4	$C_{12}H_{21}O_5Si$	±0	
247.1367	6.4	$C_{11}H_{23}O_4Si$	+0.0001	
229.1256	5.4	$C_{11}H_{21}O_3Si$	−0.0004	
159.0843	51	$C_7H_{15}O_2Si$	+0.0002	$(CH_3)_3SiO\overset{4''}{C}H=C\overset{2''}{H}C\overset{+}{H}=OCH_3$
88.0526	100	$C_4H_8O_2$	+0.0002	$CH_3O\overset{3''}{C}H=\overset{2''}{C}HOCH_3^+$

11.1 and 14.2 Hz). So the cyclic carbonate can be either at C-3′ or C-2′; but since 2′-H appears at δ 3.47 as dd (J=7.7 and 2.1 Hz) coupled to 1′-H and 2′-OH, the carbonate ring must be attached to C-3′. Fig. 4 shows protons of the ^1H NMR spectrum expanded in the region where some of the aldgarose hydrogens appear and their splitting patterns are quite discernable.

Swalpamycin has, therefore, a 16-membered lactone with a D-mycinose attached to 14-CH_2 and a D-aldgarose at C-5 positions as represented by structure **1**. This is in complete agreement with the mass spectral fragmentation observed for its trimethylsilyl derivative (**2**). The principal fragmentations are illustrated in Fig. 5 and the elemental formulae of all the ions were confirmed by high resolution mass measurements (Table 4). Fragmentations occur mainly at the glycosidic linkages except for the characteristic cleavage in the mycinose ring giving rise to a fragment of *m/z* 723 having the elemental composition $C_{30}H_{41}O_{11}$ $(Si(CH_3)_3)_2$. This in turn produces two ions of *m/z* 678 (723−COOH) and 606 (723−$COOSi(CH_3)_3$). Analogous fragmentation involving loss of C-1 and the ring oxygen accompanied by trimethylsilyl migration has been reported in the EI-MS of the trimethylsilyl ether of methylglucopyranosides[7,8]. For both the glycosidic oxygens cleavage occurs at both sides, however no glycosidic ion containing mycinose could be recognized indicating that the aglycone-mycinose linkage is particularly prone to cleavage[9]. Cleavage across the C-1″ and glycosidic oxygen bond gives rise to fragmentation of *m/z* 621 and that across 14-CH_2 and oxygen produces ion of *m/z* 605, the latter cleavage being more prevalent as evidenced by the higher intensity. Two fragments of *m/z* 333 and 317 are derived from the aglycone and they are of comparable intensities indicating that cleavage at both sides of the glycosidic oxygen of aldgarose occurs with approximately equal ease. Diagnostic fragments for the trimethylsilylated sugars appeared at *m/z* 247 for mycinose and 273 for aldgarose, the latter giving rise to an ion of *m/z* 229 *via* decarboxylation. The base peak in the mass spectrum has an *m/z* of 88 corresponding to the fragment $CH_3OCH=CHOCH_3$ obtained from the mycinose sugar unit.

Swalpamycin is thus a new addition to the plethora of 16-membered macrolide antibiotics. Its aglycone which we have named swalpanolide, is novel and occurrence of aldgarose, a relatively uncommon octose sugar is particularly interesting. Stereochemical assignments of the aglycone and of C-7′ are yet to be established.

Experimental

Melting points are determined using a Bristoline instrument and are uncorrected. UV spectra were recorded on a Uvikon 810 double beam spectrophotometer. IR spectra were recorded on a Perkin-Elmer 157 sodium chloride spectrometer and optical rotations on a Perkin-Elmer 141 polarimeter. ^{1}H NMR (90 MHz) and ^{13}C NMR (22.5 MHz) spectra were recorded on a Jeol FX 90Q instrument. High field NMR spectra were recorded on Bruker AM 400 WB spectrometer. MS were obtained using AEI MS-902 S instrument equipped with an on-line data system DS-50 SH and silylation was carried out with trifluoro bis-(trimethylsilyl)acetamide. COSY spectrum was recorded with the standard pulse scheme $\pi/2$-t_1-$\pi/2$-t_2 ($\pi/2$=6.4 μs) and phase cycling of both rf-pulses[10]. The data matrix of 1,024×2,048 points resulted from 1,024 spectra, 4K each, with a maximum acquisition time of 337.9 mseconds in t_1, and 675.8 mseconds in t_2.

Acknowledgments

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