Supporting Information

Mapping of the Primary Mannose-Binding Site of Pradimicin A

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Contents

I. Full results of feeding experiments with [2-^{13}C]AcONa and L-[5-$^{13}CH_3$]methionine

Detailed procedures of fermentation, harvesting, and purification of ^{13}C-enriched PRM-As are described in Experimental Section. The ^{13}C-population was calculated by solution ^{1}H-NMR on the basis of integration values of proton signals split with ^{1}H-^{13}C coupling.

[2-^{13}C]AcONa feeding

Feeding	Incubation time (day)						Isolation	% atom
schedule	0	1	2	3	4	6	yield	^{13}C
1	100 mg	100 mg	100 mg	100 mg	100 mg	Harvest	6.9 mg	< 2
2	50 mg	50 mg	50 mg	50 mg	50 mg		16.0 mg	ca. 20

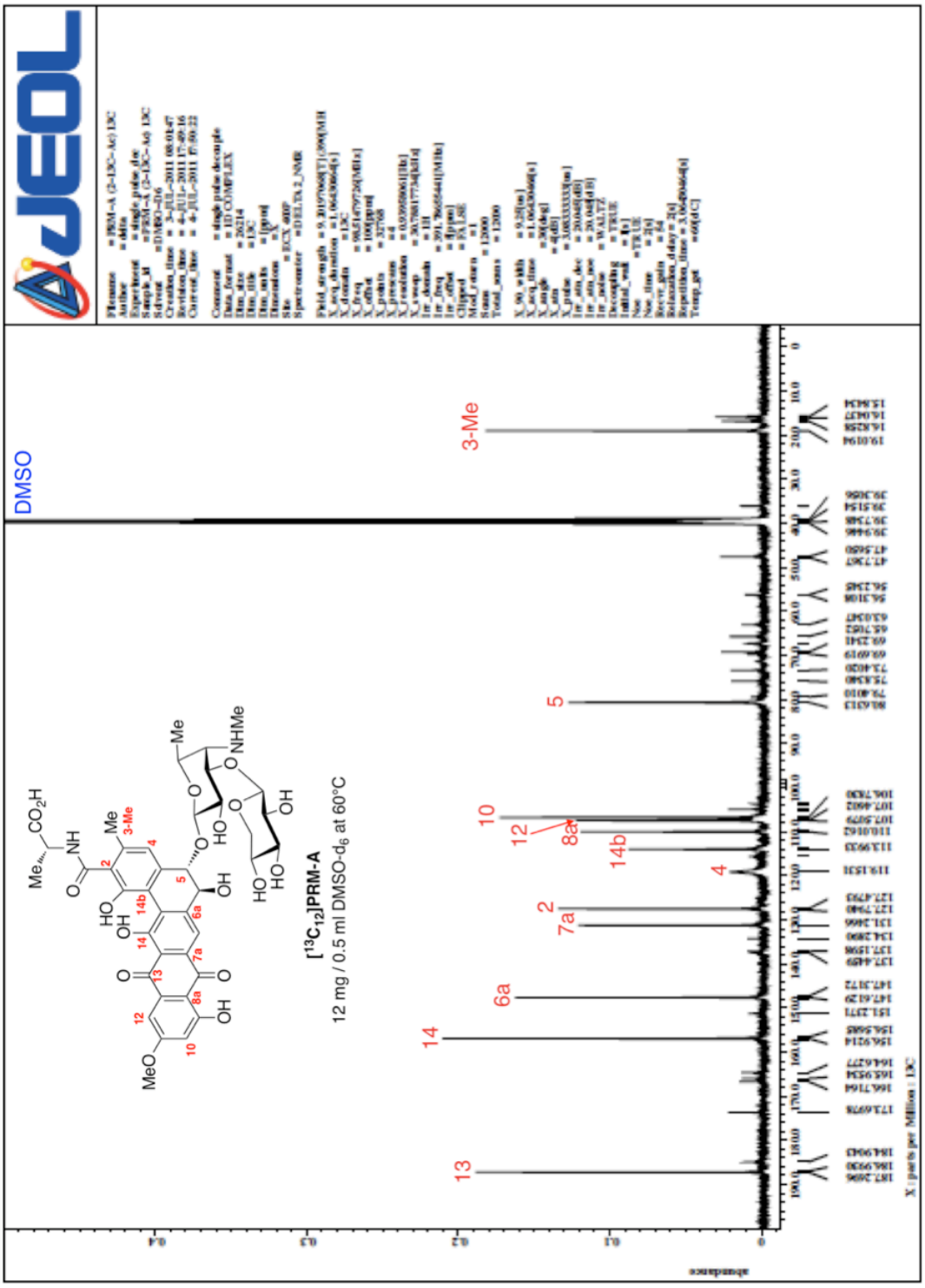
L-[5-$^{13}CH_3$]methionine feeding

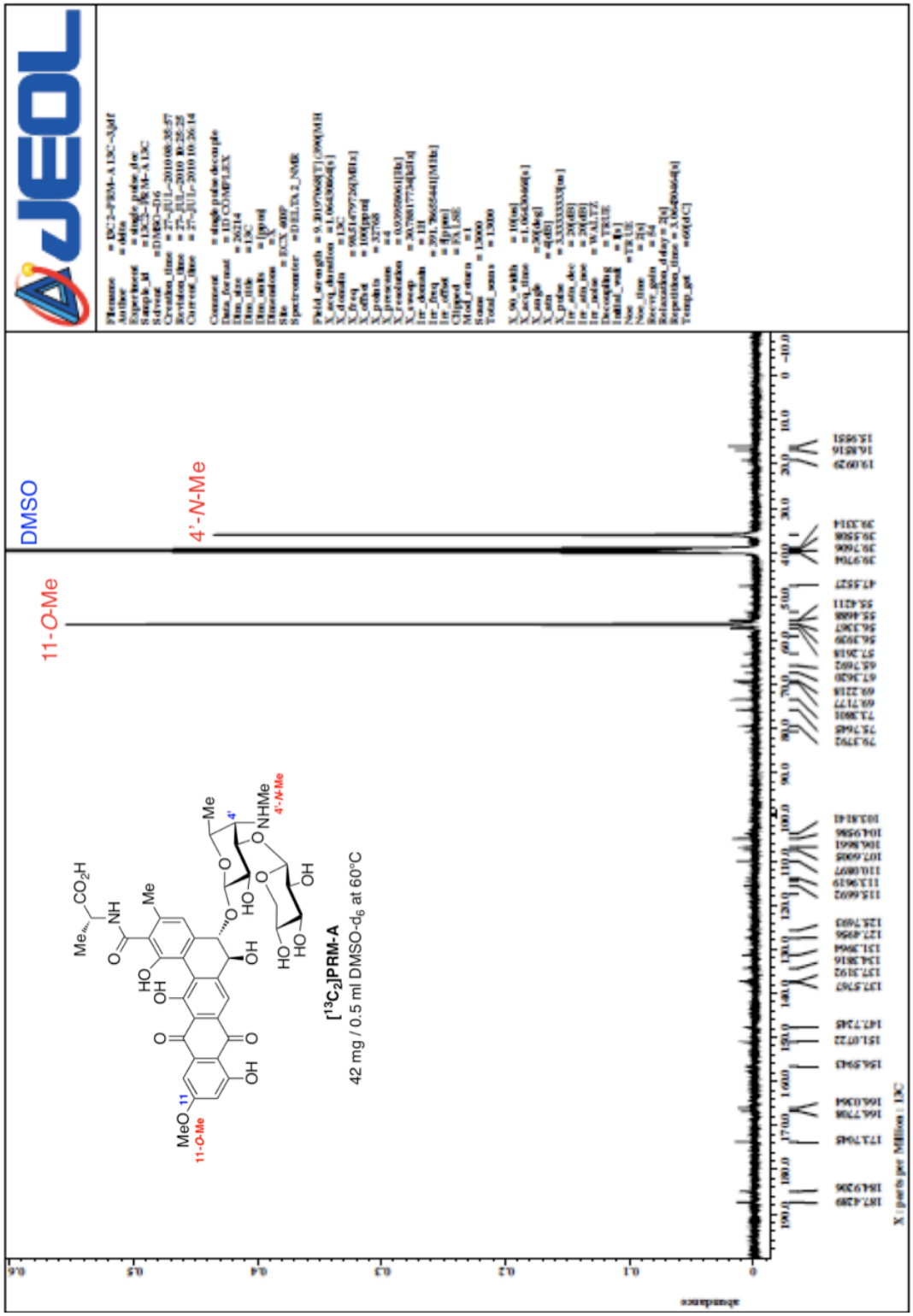
Feeding	Incubation time (day)					Isolation	% atom
schedule	0	1	2	3	6	yield	^{13}C
1	100 mg	–	–	–	Harvest	22.9 mg	ca. 45
2	100 mg	50 mg	–	–		11.0 mg	ca. 55
3	100 mg	50 mg	50 mg	–		11.1 mg	ca. 60
4	100 mg	50 mg	50 mg	50 mg		11.7 mg	ca. 65

II. Solution ^{13}C-NMR spectra of [$^{13}C_{12}$]- and [$^{13}C_2$]PRM-As

Solution ^{13}C-NMR spectra of [$^{13}C_{12}$]PRM-A (ca. 20 atom % ^{13}C) and [$^{13}C_2$]PRM-A (ca. 65 atom % ^{13}C) were obtained in DMSO-d_6 at 60°C on JEOL ECX 400 spectrometer at 100 MHz. Chemical shifts were recorded in ppm using a center peak of DMSO-d_6 (39.5 ppm) as the internal reference. Signal assignment was performed on the basis of the previous data of non-labeled PRM-A,[1] and confirmed by HMQC and HMBC spectra.

[1]Tsunakawa, M.; Nishio, M.; Ohkuma, H.; Tsuno, T.; Konishi, M.; Naito, T.; Oki, T.; Kawaguchi, H. *J. Org. Chem.* **1989**, *54*, 2532-2536.


DMSO
3-Me
5
10
12
8a
14b
4
2
7a
6a
14
13
[13C12]PRM-A
12 mg / 0.5 ml DMSO-d6 at 60°C
X : parts per Million : 13C


DMSO
11-O-Me
4'-N-Me
[13C2]PRM-A
42 mg / 0.5 ml DMSO-d6 at 60°C
X : parts per Million : 13C

III. Signal assignment of the [$^{13}C_{12}$]PRM-A_2/Ca^{2+}/[$^{13}C_6$]Man-OMe_2 complex

The ^{13}C signals of the [PRM-A_2/Ca^{2+}/Man-OMe_2] complex using [$^{13}C_{12}$]PRM-A and [$^{13}C_6$]Man-OMe were assigned on the basis of solution ^{13}C-NMR spectrum of [$^{13}C_{12}$]PRM-A and intramolecular cross peaks in 2D-DARR spectra of the complex using non-labeled Man-OMe. Soild-state 1D-^{13}C-NMR spectra (Figure S1) and 2D-DARR spectra of the complex using non-labeled Man-OMe (Figure S2) are shown below. The ^{13}C signals in the range of 101 to 108 ppm could not be assigned due to signal overlapping and the absence of intramolecular cross peaks in 2D-DARR spectra.

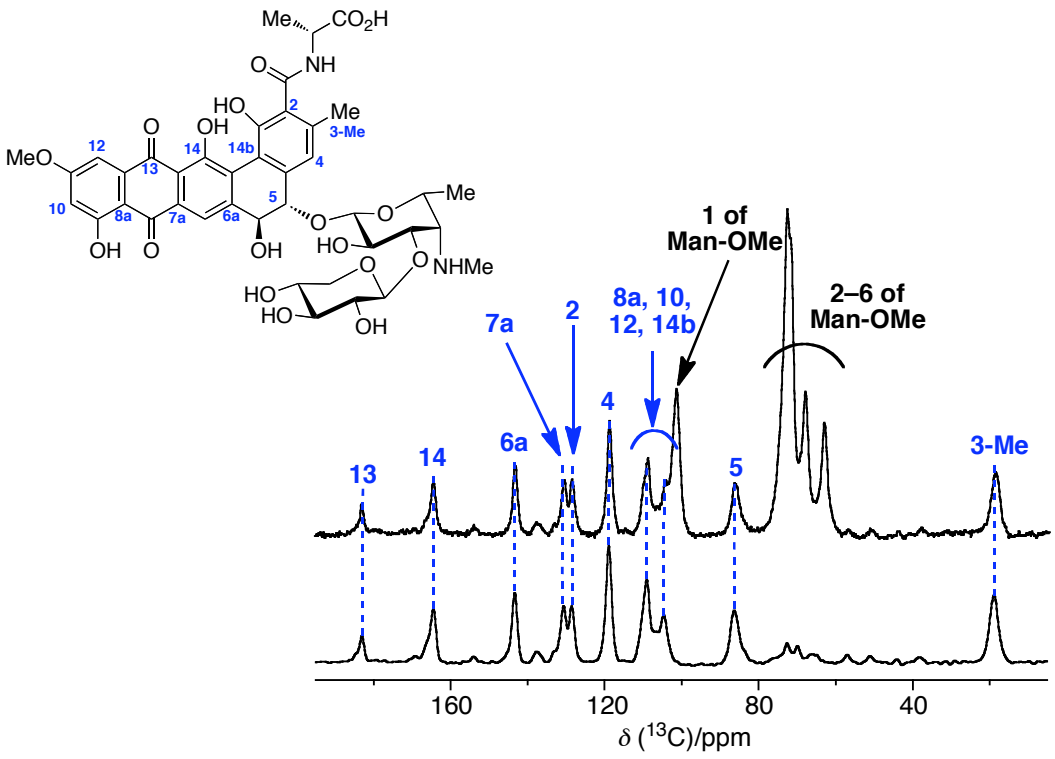


Figure 1S. Solid-state 1D-^{13}C-NMR spectra of the [PRM-A_2/Ca^{2+}/Man-OMe_2] complexes using [$^{13}C_{12}$]PRM-A and [$^{13}C_6$]Man-OMe (upper) or non-labeled Man-OMe (lower).

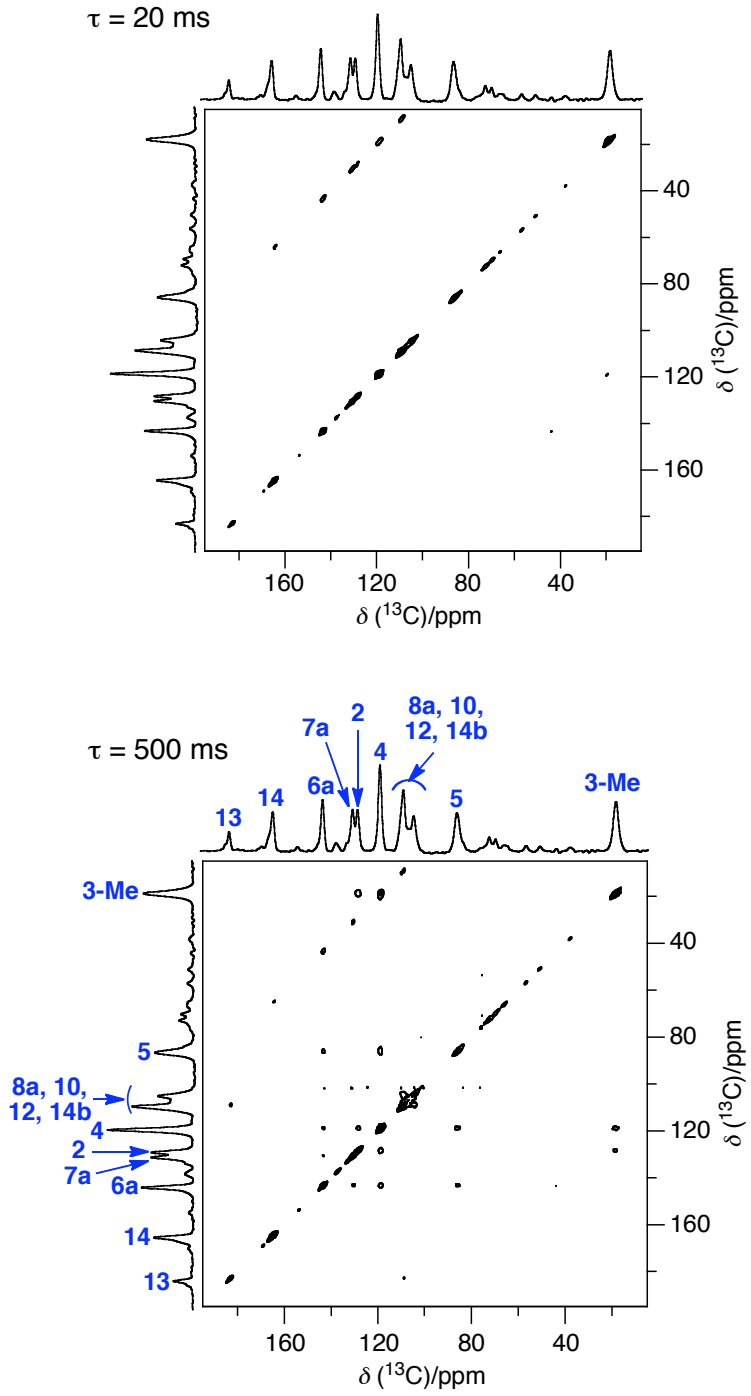


Figure 2S. 2D-DARR spectra of the [PRM-A_2/Ca^{2+}/Man-OMe_2] complexes using [$^{13}C_{12}$]PRM-A and non-labeled Man-OMe at the mixing time of 20 ms (upper) and 500 ms (lower).