Secondary Extended Mannan Side Chains and Attachment of the Arabinan in Mycobacterial Lipoarabinomannan

Shiva K. Angala¹, Wei Li¹, Claudia M. Boot², Mary Jackson¹* and Michael R. McNeil¹*

From the ¹Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, USA, and the ²Central Instrument Facility, Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA.
**Supplementary Note 1:** Preliminary control experiments to interpret the treatment of *M. smegmatis* AMSM6387 Ara-LM with \( \alpha \)-mannosidase.

In the case of Msm-Ara-LM, we expected all internal mannosyl residues in the \( \alpha \)-1,6 backbone between the T-Araf residue and the inositol to not be susceptible to \( \alpha \)-mannosidase. Thus, the enzyme removes the side chain \( \alpha \)-mannosyl residues but not those in the backbone. To rightly interpret the data, we needed to determine the susceptibility of the \( \alpha \)-Manp unit attached to O-2 of the inositol and thus a mixture of deacylated PIM\(_2\) and PIM\(_6\) were treated with \( \alpha \)-mannosidase. LC/MS analysis revealed that PIM\(_1\) was not produced, PIM\(_2\) was essentially unchanged, and PIM\(_6\) was converted to PIM\(_2\). Thus the \( \alpha \)-mannosidase does not remove mannosyl residues attached to inositol at either position 2 or 6. Therefore, the number of mannosyl residues in the \( \alpha \)-1,6 mannan chain separating the Araf from the inositol (whether or not they are substituted with an \( \alpha \)-mannosyl residue at O-2) is equal to the number of mannosyl residues in the \( \alpha \)-mannosidase treated LM minus one as the mannosyl residue on O-2 of the inositol residue is not removed. With this information, available, Msm-Ara-LM was treated with \( \alpha \)-mannosidase. To confirm the lack of branched residues in the resulting products, MS/MS analysis was conducted on the triply charged \([M-(H)3]^3^-\) ion of non-\( \alpha \)-mannosidase digested *M. smegmatis* Ara\(_1\)-Man\(_{24}\)-Inos-P-Gro (m/z 1450.78) as a control, and the triply charged \([M-(H)3]^3^-\) ion of the \( \alpha \)-mannosidase digested product Ara-Man\(_{17}\)-Inos-P-Gro (m/z 1072.66) (Supplementary Figure 7). A major contrast was noted between the two spectra, as in the \( \alpha \)-mannosidase digested product Ara-Man\(_{17}\)-Inos-P-Gro the loss of a single mannosyl (presumably from O-2 of the inositol) to form the Y ion Ara\(_1\)Man\(_{16}\)-Inos-P-Gro was only very weakly observed but the loss of the non-reducing end Araf residues to form the Y ion Man\(_{17}\)-Inos-P-Gro was abundantly formed (Supplementary Figure 7a). In contrast, the non-enzyme treated control, Ara\(_3\)Man\(_{24}\)-Inos-P-Gro (Supplementary Figure 7b) shows the opposite results where only very rarely is the Y ion Man\(_{24}\)-Inos-P-Gro formed by loss of Ara observed but the Y ion Ara-Man\(_{23}\)-Inos-P-Gro formed by loss of a single mannosyl residue is abundant. Thus, the MS/MS data confirmed that the general structure of
the α-mannosidase products produced from Msm-Ara-LM were linear molecules with the arabinosyl residue at the non-reducing terminus.

**Supplementary Data 1** (provided as a separate Excel file)
**Supplementary Figure 1:** LC/MS analysis of native deacylated Msm-Ara-LM from *M. smegmatis* ΔembC. Shown are base peak chromatograms (BPC) of Msm-Ara-LM in negative mode (a), extracted ion chromatograms (EICs) of GPI-Man$_{38}$ at $m/z$ 1622.01 [M-4H]$^4$-(b), GPI-Ara$_1$Man$_{25}$ at $m/z$ 1504.80 [M-3H]$^3$-(c), GPI-Ara$_2$Man$_{22}$ at $m/z$ 1386.76 [M-3H]$^3$-(d), and GPI-Ara$_3$Man$_{21}$ at $m/z$ 1376.76.80 [M-3H]$^3$-(e). Multiply charged spectra from Fig. 2(a-d) are deconvoluted to singly charged [M-H]$^-$ spectra (f-i). The corresponding mass accuracy values for the individual compounds are shown in Supplementary Table 1.
**Supplementary Figure 2:** LC/MS analysis of α-mannosidase and endomannanase-digested Msm-Ara-LM from *smegmatis ΔembC* in positive mode. (a) Shown are the BPCs and EICs of P2-column purified acetylated fractions. The extracted ion chromatogram (EIC) at m/z 912.29 shows the elution profile of acetylated products. (b) Mass spectrum of the peaks revealed acetylated Ara-Man-Man with the m/z values of 917.25 and 912.29 as sodiated and ammonium adducts.
Supplementary Figure 3: (a) Shows the total ion chromatogram (TIC) of methylated Ara-Man-mannitol resulting from Msm-Ara-LM of *M. smegmatis ΔMSMEG_6387* in positive mode. (b) Extracted ion chromatogram at m/z 649.38, showing a single peak with a retention time (RT) 6.79 min representing Ara-Man-mannitol. Inset shows the structure and mass spectrum of reduced (deuterated), methylated Ara-
Man-mannitol with the $m/z$ values of 654.34, and 649.38 as Na$^+$ and NH$_4^+$ adducts. Panel (c) shows TIC of *Cellulomonas* treated *M. tuberculosis* deacylated LAM after digestion with a mixture of $\alpha$-mannosidase and endo 1,6-$\alpha$-mannanase obtained in negative mode. The EICs of $m/z$ 473.15 and 605.19 shown in panel (c) correspond to the non-derivatized trisaccharide and tetrasaccharide. Their mass spectra of [M-H]$^-$ ions are shown in panel (d).
Supplementary Figure 4: GC/MS analysis of the partially O-methylated partially O-acetylated alditols derived from the Ara$_1$Man$_2$ trisaccharide isolated from enzymatic degradation of Msm-Ara-LM produced by *M. smegmatis* ΔMSMEG_6387. (a) Peaks 1, 4, 5, 6, and 7 labeled on the TIC are non-carbohydrate contaminants (due to the low amounts of trisaccharide analyzed), and peaks 2, 3, and 8 are the partially-O-methylated partially-O-acetylated alditols. (b) The two hexitols were shown to be in the manno-configuration by comparison with standards. (c) The MS spectra of the 1,4-di-O-acetyl 2,3,5-tri-O-methyl arabinitol, 6-O-acetyl 1,2,3,4,5 penta-O-methyl mannitol and 1,5,6-tri-O-acetyl 2,3,4-tri-O-methyl mannitol.
Supplementary Figure 5: LC/MS analysis of Cellulomonas endo-arabinanase digested native, deacylated M. tuberculosis LAM. (a) Shown are the BPC, and EIC obtained in the negative mode for the triply charged dominant ion at $m/z$ 1602.83 [M-3H]$^3$-. The EIC generated represents the GPI-Ara$_2$Man$_{26}$ show in Fig. 4. (b) Deconvoluted mass spectrum obtained at 0.3 min retention time show dominant signals for Ara$_2$Manx-Gro in the digestion mixture. Mass accuracy (ppm) values for the corresponding deconvoluted spectrum are shown in Supplementary Table 3.
Supplementary Figure 6: (Panel a) LC-MS/MS spectrum of triply charged \((\text{NH}_4)^{3+}\) per-\(O\)-methylated Ara\(_1\)-Man\(_{23}\)-Inos-P-Gro at \(m/z\) 1784.89. The interpretation of observed singly charged fragment ions (most abundant) is presented in the inset structure. The vertical dotted arrows mark the low mass oxonium ions. Panel (b) shows a mass spectrum for all low mass oxonium ions identified, demonstrating the clear fragmentation pattern of the molecule at the site of arabinose attachment on the mannan backbone. Assignments of fragment oxonium ions are illustrated as insets (b).
Supplementary Figure 7: A comparison of the loss of mannosyl versus arabinosyl residues in the MS/MS spectra on the triple charged \([M-(H)_3]^3+\) ions (a) at \(m/z\) 1072.66 of underivatized Ara\(_1\)Man\(_{18}\)-Inos-P-Gro produced by \(\alpha\)-mannosidase treatment of Msm-Ara-LM from \textit{M. smegmatis} Δ\textit{MSMEG}_6387 and (b) at \(m/z\) 1450.78 of underivatized Ara\(_1\)Man\(_{24}\) Inos-P-Gro from \textit{M. smegmatis} Δ\textit{MSMEG}_6387 that was not treated with \(\alpha\)-mannosidase. (See Supplemental data 1 for more details). Interpretation of observed fragments ions by single cleavage are illustrated as insets.
Supplementary Figure 8: LC/MS analysis showing base peak chromatograms of the α-mannosidase digestion products from (a) Msm-Ara-LM, and (b) Cellulomonas endo-arabinanase-digested M. tuberculosis LAM. Their corresponding mass spectra are obtained in negative mode (c-d). Mass spectrum
from (c) Msm-Ara-LM shows the presence of triply charged ions [M-3H]^{3-} for 13-18 mannosyl residues between the non-reducing end of arabinose and inositol. Mass spectrum from (d) *Cellulomonas* endo-arabinanase digested *M. tuberculosis* LAM shows a series of triply charged ions [M-3H]^{3-} for 15-25 mannosyl residues between the non-reducing end of terminal arabinose and inositol.
Supplementary Figure 9: A low mass portion of the MS/MS spectrum of the triple charged [M+(NH₄)₃]⁺ ion at m/z 1784.8 produced by the per-O-methylated Ara₁Man₂₃-Inos-P-Gro molecule by *M. smegmatis ΔMSMEG_6387* showing diagnostic B ions and the rationale for their formation.

(a) m/z 848.44 made up of 4 mannosyl residues, (b) m/z 1052.56 made up of 5 mannosyl residues, and (c) m/z 1256.64 made up of 6 mannosyl residues. These mannosyl residues are shown in a linear arrangement for clarity, but the actual arrangement of the mannosyl residues (*i.e.*, branching pattern) is not yet known. These B ions, although of low intensity, confirm the extended mannan backbone side chains. The peaks with an asterisk are from other unidentified ions.
Supplementary Figure 10: BPCs and EICs (m/z 1906.27) of the per-O-methylated Ara2Man24-Inos-P-Gro molecule produced by *Cellulomonas*-digested *M. tuberculosis*-LAM (a) and *M. smegmatis ΔMSMEG_6387* (c) were obtained in positive mode. Panels b and d shows their corresponding MS spectra of the triple charged [M+(NH₄)₃]³⁺ ion at m/z value of 1906.27.
Supplementary Table 1. Assignment of major ionic species found in the complex mixture of Msm-Ara-LM from *M. smegmatis ΔMSMEG_6387* (see Fig 2a-d).

| Glycerol phosphate-inositol (GPI)- | Charge | Theoretical mass (m/z) | Observed mass (m/z) | Mass error (Da) | Mass accuracy (ppm) |
|-----------------------------------|--------|------------------------|---------------------|-----------------|---------------------|
| with varying number of |        |                        |                     |                 |                     |
| mannosyl/arabinosyl residues on |        |                        |                     |                 |                     |
| the mannan backbone              |        |                        |                     |                 |                     |
| GPIM32 GPIM33                     | [M-H+] | 1419.4641              | 1419.4648           | 0.002           | 1.2                 |
| GPIM34 GPIM35                     | [M-H+] | 1459.9583              | 1459.9641           | 0.006           | 4.0                 |
| GPIM36 GPIM37                     | [M-H+] | 1500.4715              | 1500.4797           | 0.008           | 5.4                 |
| GPIM38 GPIM39                     | [M-H+] | 1540.9847              | 1540.9903           | 0.006           | 3.6                 |
| GPIM40 GPIM41                     | [M-H+] | 1591.4979              | 1591.5005           | 0.006           | 4.8                 |
| GPIM42 GPIM43                     | [M-H+] | 1622.0111              | 1622.0212           | 0.010           | 6.2                 |
| GPIM44 GPIM45                     | [M-H+] | 1662.5243              | 1662.5288           | 0.004           | 2.7                 |
| GPIM46 GPIM47                     | [M-H+] | 1703.0375              | 1703.0424           | 0.005           | 2.8                 |
| GPIM48 GPIM49                     | [M-H+] | 1743.5508              | 1743.5667           | 0.008           | 3.4                 |
| GPIM50 GPIM51                     | [M-H+] | 1784.0640              | 1784.0860           | 0.002           | 1.1                 |
| GPIM52 GPIM53                     | [M-H+] | 1824.5772              | 1824.5951           | 0.018           | 9.8                 |
| GPIM54 GPIM55                     | [M-H+] | 1865.0904              | 1865.1028           | 0.010           | 5.6                 |
| GPIM56 GPIM57                     | [M-H+] | 1905.6036              | 1905.6057           | 0.002           | 1.1                 |
| GPIM58 GPIM59                     | [M-H+] | 1946.1168              | 1946.1314           | 0.015           | 7.5                 |
| GPIM60 GPIM61                     | [M-H+] | 1986.6300              | 1988.6356           | 0.006           | 2.8                 |
| GPIM62 GPIM63                     | [M-H+] | 2027.1432              | 2027.1603           | 0.017           | 8.4                 |

* The ions identified in Fig 2a.

* The ions identified in Fig 2b.

* The ions identified in Fig 2c.

* The ions identified in Fig 2d.
### Supplementary Table 2: Structural assignment of MS/MS fragment ions for the reduced-methylated Ara-Man-mannitol (positive mode), native underivatized tri-Ara-Man-Man and tetra Ara-Ara-Man-Man (negative mode) from Fig. 3(a-c).

| Ara1-Man2-Reduced-Methylated<sup>a</sup> | MS/MS Fragment ions | Charge | Theoretical mass (m/z) | Observed mass (m/z) | Mass error (Da) | Mass accuracy (ppm) |
|-----------------------------------------------|----------------------|--------|-------------------------|---------------------|----------------|---------------------|
| B<sub>1</sub> | [M+Na]<sup>+</sup> | 197.0784 | 197.0769 | -0.001 | -7.61 |
| B<sub>2</sub> | [M+Na]<sup>+</sup> | 401.1782 | 401.1681 | -0.010 | -25.18 |
| C<sub>1</sub> | [M+Na]<sup>+</sup> | 215.0890 | 215.0903 | 0.001 | 6.04 |
| C<sub>2</sub> | [M+Na]<sup>+</sup> | 419.1888 | 419.1884 | 0.000 | -0.95 |
| Y<sub>1</sub> | [M+Na]<sup>+</sup> | 276.1528 | 276.1535 | 0.001 | 2.53 |
| Y<sub>2</sub> | [M+Na]<sup>+</sup> | 480.2526 | 480.2548 | 0.002 | 4.58 |
| A<sub>2</sub><sup>0.4</sup> | [M+Na]<sup>+</sup> | 257.0996 | 257.0650 | -0.035 | -134.58 |
| A<sub>2</sub><sup>3.5</sup> | [M+Na]<sup>+</sup> | 285.1309 | 285.1376 | 0.007 | 23.50 |
| A<sub>2</sub><sup>3.5</sup> | [M+Na]<sup>+</sup> | 489.2306 | 489.2347 | 0.004 | 8.38 |

| Ara1-Man2 underivatized<sup>b</sup> | MS/MS Fragment ions | Charge | Theoretical mass (m/z) | Observed mass (m/z) | Mass error (Da) | Mass accuracy (ppm) |
|-----------------------------------------------|----------------------|--------|-------------------------|---------------------|----------------|---------------------|
| B<sub>1</sub> | [M-H] | 131.0350 | 131.0356 | -0.001 | -4.58 |
| C<sub>1</sub> | [M-H] | 149.0455 | 149.0467 | -0.001 | -8.05 |
| C<sub>2</sub> | [M-H] | 311.0984 | 311.1003 | -0.002 | -6.11 |
| A<sub>2</sub><sup>0.4</sup> | [M-H] | 191.0561 | 191.0573 | -0.001 | -6.28 |
| A<sub>2</sub><sup>0.3</sup> | [M-H] | 221.0667 | 221.0681 | -0.001 | -6.33 |
| A<sub>2</sub><sup>0.2</sup> | [M-H] | 251.0772 | 251.0789 | -0.002 | -6.77 |
| A<sub>2</sub><sup>0.4</sup> | [M-H] | 353.1089 | 353.1109 | -0.002 | -5.66 |
| A<sub>2</sub><sup>0.2</sup> | [M-H] | 413.1301 | 413.1312 | -0.001 | -2.66 |

| Ara2-Man2 underivatized<sup>c</sup> | MS/MS Fragment ions | Charge | Theoretical mass (m/z) | Observed mass (m/z) | Mass error (Da) | Mass accuracy (ppm) |
|-----------------------------------------------|----------------------|--------|-------------------------|---------------------|----------------|---------------------|
| C<sub>1</sub> | [M-H] | 149.0455 | 149.0453 | 0.000 | 1.34 |
| C<sub>2</sub> | [M-H] | 281.0878 | 281.0886 | -0.001 | -2.85 |
| C<sub>3</sub> | [M-H] | 443.1406 | 443.1415 | -0.001 | -2.03 |
| A<sub>2</sub><sup>0.3</sup> | [M-H] | 191.0561 | 191.0569 | -0.001 | -4.19 |
| A<sub>2</sub><sup>0.3</sup> | [M-H] | 323.0984 | 323.0984 | 0.000 | 0.00 |
| A<sub>2</sub><sup>0.3</sup> | [M-H] | 353.1089 | 353.1085 | 0.000 | 1.13 |
| A<sub>3</sub><sup>0.2</sup> | [M-H] | 383.1195 | 383.1199 | 0.000 | -1.04 |
| A<sub>4</sub><sup>0.2</sup> | [M-H] | 485.1512 | 485.1513 | 0.000 | -0.21 |
| A<sub>4</sub><sup>0.2</sup> | [M-H] | 545.1723 | 545.1722 | 0.000 | 0.18 |

<sup>a</sup> The ions identified in Fig. 3a  
<sup>b</sup> The ions identified in Fig. 3b  
<sup>c</sup> The ions identified in Fig. 3c
Supplementary Table 3. Assignment of major ionic species found in the complex mixture of *Cellulomonas* endo-arabinanase digested native, decacylated *M. tuberculosis* LAM.

| Glycerol phosphate-Inositol (GPI)- | Theoretical mass (m/z) | Observed mass (m/z) | Mass error (Da) | Mass accuracy (ppm) |
|-----------------------------------|------------------------|---------------------|----------------|-------------------|
| with varying number of mannosyl | [M-H]                  | [M-H]               |                |                   |
| residues in the mannan backbone  |                        |                     |                |                   |
| G1-P21                           | 3706.15789             | 3706.2075           | 0.050         | 13.39             |
| G1-P21                           | 3868.21071             | 3868.2546           | 0.044         | 11.36             |
| G1-P22                           | 4030.26535             | 4030.3085           | 0.045         | 11.16             |
| G1-P23                           | 4192.31635             | 4192.3663           | 0.050         | 11.91             |
| G1-P24                           | 4534.36917             | 4534.4082           | 0.039         | 8.96              |
| G1-P25                           | 4516.42199             | 4516.4617           | 0.040         | 8.79              |
| G1-P26                           | 4678.47481             | 4678.5243           | 0.049         | 10.58             |
| G1-P27                           | 4840.52763             | 4840.5771           | 0.049         | 10.22             |
| G1-P28                           | 5002.58045             | 5002.6271           | 0.047         | 9.35              |
| G1-P29                           | 5164.63327             | 5164.6787           | 0.045         | 8.80              |
| G1-P30                           | 5326.68609             | 5326.7313           | 0.045         | 8.49              |
| G1-P31                           | 5488.73991             | 5488.7878           | 0.059         | 10.73             |
| G1-P32                           | 5650.79173             | 5650.8497           | 0.058         | 10.26             |
| G1-P33                           | 5812.84455             | 5812.9088           | 0.064         | 11.05             |
| G1-P34                           | 5794.89737             | 5794.9261           | 0.029         | 4.81              |
| G1-P35                           | 6136.95019             | 6137.0255           | 0.075         | 12.19             |
| G1-P36                           | 6299.00301             | 6299.0444           | 0.041         | 6.57              |
| G1-P37                           | 6461.05583             |                    |              |                   |
| G1-P38                           | 6623.10865             | 6623.1428           | 0.034         | 5.16              |
| G1-P39                           | 6785.16147             | 6785.1998           | 0.038         | 5.65              |
| G1-P40                           | 6947.21429             |                    |              |                   |
| G1-P41                           | 7109.26713             | 7109.3309           | 0.064         | 8.97              |
| G2-P1                            | 3352.04169             | 3352.0776           | 0.036         | 10.71             |
| G2-P1                            | 3514.09451             | 3514.1285           | 0.033         | 9.53              |
| G2-P1                            | 3676.14733             | 3676.2009           | 0.054         | 14.57             |
| G2-P2                            | 3838.20915             |                    |              |                   |
| G2-P2                            | 4000.25297             |                    |              |                   |
| G2-P2                            | 4162.30579             | 4162.3511           | 0.045         | 10.89             |
| G2-P2                            | 4324.35861             | 4324.4047           | 0.046         | 10.66             |
| G2-P2                            | 4486.41433             | 4486.4635           | 0.052         | 11.61             |
| G2-P2                            | 4648.46425             | 4648.5049           | 0.041         | 8.74              |
| G2-P2                            | 4810.51707             | 4810.5547           | 0.038         | 7.82              |
| G2-P2                            | 4972.56989             | 4972.6001           | 0.030         | 6.08              |
| G2-P2                            | 5134.62271             | 5134.6659           | 0.043         | 8.41              |
| G2-P2                            | 5296.67553             | 526.71113           | 0.036         | 6.72              |
| G2-P2                            | 5458.72835             | 5458.7807           | 0.052         | 9.59              |
| G2-P2                            | 5620.78117             | 5620.8397           | 0.059         | 10.41             |
| G2-P2                            | 5782.83993             | 5782.8893           | 0.055         | 9.56              |
| G2-P2                            | 5944.88681             | 5944.9302           | 0.043         | 7.30              |
| G2-P2                            | 6106.93963             | 6106.9999           | 0.059         | 9.72              |
| G2-P2                            | 6268.99245             | 6269.0302           | 0.038         | 6.02              |
| G2-P2                            | 6431.04527             | 6431.0945           | 0.049         | 7.66              |
| G2-P2                            | 6593.09809             | 6593.1743           | 0.076         | 11.56             |
| G2-P2                            | 6755.15991             |                    |              |                   |

* a, b, & c The ions obtained after deconvolution of mass spectrum from Fig 4.
**Supplementary Table 4.** Assignment of fragment ions from the MS/MS analysis of the per-\(O\)-methylated Ara\(_2\)-Man\(_{24}\)-Inos-P-Gro at \(m/z\) 1906.27 \([M+(NH_4)_3]^3+\) (see Fig. 9).

| Varying number of Mannosyl residues | Single Cleavage Y-ions | Charge Status \((NH_4^+\) adduct) | Theoretical m/z values | Observed m/z values | Mass accuracy (ppm) | Observed m/z values | Mass accuracy (ppm) |
|-------------------------------------|------------------------|----------------------------------|------------------------|---------------------|---------------------|---------------------|---------------------|
| Ara2Man20                           | Y20-(OH)\(_1\)         | 2                                | 2435.1931              | 2435.6884           | 203.4               | 2435.1858           | -3.0                |
| Ara2Man21                           | Y21-(OH)\(_1\)         | 2                                | 2537.243               | 2537.2352           | -3.1                | 2537.2458           | 1.1                 |
| Ara2Man22                           | Y22-(OH)\(_1\)         | 2                                | 2639.2929              | 2639.2932           | 0.1                 | 2639.286            | -2.6                |
| Ara2Man23                           | Y23-(OH)\(_1\)         | 2                                | 2741.3428              | 2741.3298           | -4.7                | 2741.3553           | 4.6                 |
| Ara2Man19                           | Y19-(OH)\(_2\)         | 2                                | 2326.1354              | 2326.6331           | 214.0               | 2326.1175           | -7.7                |
| Ara2Man20                           | Y20-(OH)\(_2\)         | 2                                | 2428.1853              | 2428.1723           | -5.3                | 2428.1818           | -1.4                |
| Ara2Man21                           | Y21-(OH)\(_2\)         | 2                                | 2530.2351              | 2530.2395           | 1.7                 | 2530.2378           | 1.1                 |
| Ara2Man22                           | Y22-(OH)\(_2\)         | 2                                | 2632.285               | 2632.2897           | 1.8                 | 2632.2762           | -3.4                |
| Ara1Man20                           | Y20-(OH)\(_1\)         | 2                                | 2355.1563              | nd                  | _                   | 2355.1635           | 3.0                 |
| Ara1Man21                           | Y21-(OH)\(_1\)         | 2                                | 2457.2062              | nd                  | _                   | 2457.2145           | 3.4                 |
| Ara1Man22                           | Y22-(OH)\(_1\)         | 2                                | 2559.2561              | nd                  | _                   | 2559.267            | 4.3                 |

nd-not detected  
* The ions identified in Fig. 9