



- (51) **International Patent Classification:**
C12Q 1/04 (2006.01) *G01N 27/00* (2006.01)
- (21) **International Application Number:**
PCT/SG2012/000034
- (22) **International Filing Date:**
7 February 2012 (07.02.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/440,908 9 February 2011 (09.02.2011) US
61/550,803 24 October 2011 (24.10.2011) US
- (71) **Applicant (for all designated States except US):** NATIONAL UNIVERSITY OF SINGAPORE [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** WENK, Markus R [CH/SG]; c/o National University of Singapore, Yong Loo Lin School of Medicine, Department of Biochemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG). SHUI, Guanghou [CN/SG]; c/o National University of Singapore, Yong Loo Lin School of Medicine, Department of Biochemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG). BENDT, Anne Kathrin [DE/SG]; c/o National University of Singapore, Yong Loo Lin School of Medicine, Department of Biochemistry, 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(74) **Agent:** AMICA LAW LLC; 30 Raffles Place, #18-03/04 Chevron House, Singapore 048622 (SG).

(81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

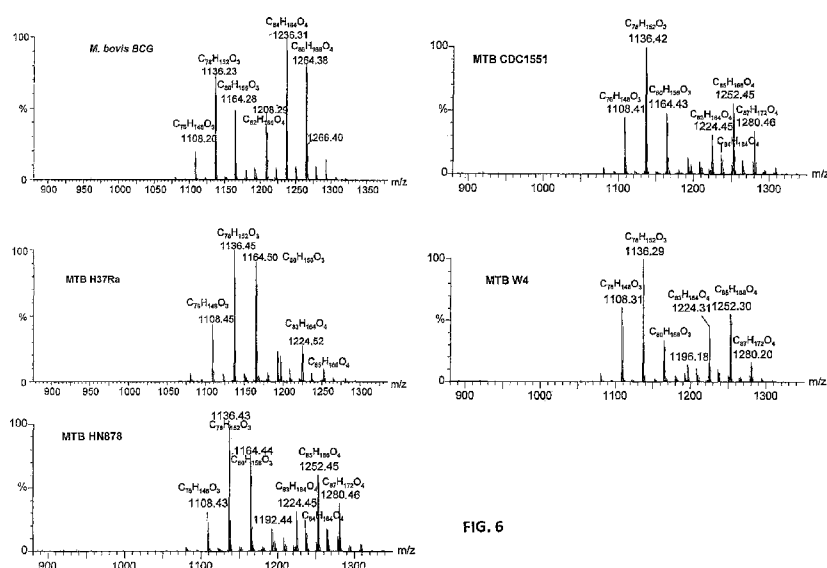
Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) **Title:** MYCOLIC ACIDS AS DIAGNOSTIC MARKERS FOR TUBERCULOSIS CASE DETECTION AND DRUG EFFICACY



MYCOLIC ACIDS AS DIAGNOSTIC MARKERS FOR TUBERCULOSIS CASE DETECTION AND DRUG EFFICACY

BACKGROUND OF THE INVENTION

The number of mycobacterial infections has increased dramatically over the
5 past two decades due to, among other factors, the HIV/AIDS epidemic. Infection
with *Mycobacterium tuberculosis* (MTB) remains a major global health threat, with
over nine million new cases and close to two million deaths annually. Detection of
active tuberculosis (TB) infection remains a serious problem in areas where TB is
present and in pre-clinical and clinical trials. Furthermore, there are currently no
10 markers available that reliably reflect drug response and clearance of bacterial
remnants, for example from lesions in the lung.

SUMMARY OF THE INVENTION

The methods of the invention are drawn to methods of assessing a test
15 sample for the presence of *Mycobacterium tuberculosis* by assessing a profile of
mycolic acids, such as C26- α -mycolic acids, in a test sample using electrospray
ionization mass spectrometry. The presence of a profile of mycolic acids
comparable to a profile in a positive sample, indicates the presence of
Mycobacterium tuberculosis and is diagnostic for active tuberculosis infection. The
20 methods further comprise methods for assessing efficacy of a treatment for
tuberculosis, as the presence of a profile of mycolic acids in a test sample from an
individual after treatment that is comparable to the profile of mycolic acids in a
positive sample is indicative of the continued presence of *Mycobacterium*
tuberculosis, and thus of a lack of efficacy or of a need for further treatment. A
25 profile in a test sample from an individual after treatment that is not comparable to
the profile in a positive sample is indicative of an absence of detectable
Mycobacterium tuberculosis, and therefore of the efficacy of the treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

Figs. 1A-1E depict mass spectrometry and fingerprints of mycolic acids (MAs). MAs from bacterial cell walls were extracted and analyzed by electrospray ionization mass spectrometry (ESI/MS) in negative mode. Fig. 1A: ESI/MS spectrum of MAs derived from *M. tuberculosis* Beijing strain. Fig. 1B: product ion analysis (MS/MS) of m/z 1,164, indicating the presence of C22:0 (m/z 339), C24:0 (m/z 367) and C26:0 (m/z 395) fatty acyls in the alpha-branch. (See Fig. 5-12 for additional ESI/MS of MAs derived from *Corynebacteria*, *Nocardia* and *Mycobacteria*.) Fig 1C: Signal intensity for each MA species is expressed in a heat plot format; the inset shows an enlarged section of the alpha-MAs in nine mycobacterial strains. The triangle indicates a major alpha-MA $C_{80}H_{156}O_3$ with C26 as the alpha-branch. The asterisk indicates the alpha-MA $C_{76}H_{148}O_3$ with C24 as the alpha-branch. The full list of all 1,942 MAs is described in Table S1. Fig. 1D: single stage mass spectra of sputum from non-TB control and from TB patient (ESI/MS), with ion counts indicated as counts per second (CPS). Fig. 1E: limit of detection of MA in sputum and in media, using serial dilutions of *M. tuberculosis* spiked into control sputum and medium. After MA extraction, the MA were resuspended in 120 μ L mobile phase and quantified by MRM and normalization to internal MA standard C32. Ion responses for both medium and sputum are shown using a non-linear polynomial regression curve.

Figs. 2A-2F demonstrate that mycolic acids (MAs) in sputum are diagnostic for TB infection in humans. Fig. 2A: diagram illustrating case-control set-up and number of TB patients ($n=70$) and non-TB controls ($n=40$). Fig. 2B: sputum samples were extracted using organic solvents and levels of individual MA molecular species were determined using mass spectrometry in multiple reaction monitoring (MRM) mode. Major MA species for alpha-, M- (methoxy-) and K-

(keto-) MA with their respective molecular composition are plotted, with (24) and (26) indicating the number of carbon atoms in their respective alpha chains. Fig. 2C: individual MA molecular species varied in the strength of their differentiation between non-TB controls and TB patients. Fig. 2D: receiver operating characteristic (ROC) curve is shown displaying the classifying performance (positive diagnostic likelihood ratio) of individual MA molecular species, expressed by true positive rate (sensitivity) and false positive rate (1-specificity), with alpha-MA $C_{80}H_{156}O_3$ providing best accuracy. Fig. 2E: confusion matrix for all 110 samples. Fig. 2F: confusion matrix for the 39 HIV positive samples.

10 Figs. 3A-B show that mycolic acids (MAs) in lung tissue are diagnostic markers for TB infection in mice. Fig. 3A: levels of individual MA molecular species in the lung of one infected, untreated mouse 5 weeks post-infection. Fig. 3B: MA levels of non-infected control mice compared to MTB-infected and to rifampicin-treated mice. Average bacillary load in infections was 15.2 million
15 cfu/lung (n=5).

Fig. 4 depicts mass spectra profiles of mycolic acids (MAs) from corynebacteria. *C. hofmannii* peaks at m/z 495 and 521 for C32:0 and C34:1 acids; *C. matruchotii* peaks at m/z 495, 521, 547 for C32:0, C34:1, and C36:2 acids; *C. ovis*, one major homologue at m/z 495 for C32:0 corynomycolic acids; *C. vitarumen*
20 peaks at m/z 493, 495, 521, and 523 corresponding to C32:1, C32:0, C34:1, and C34:0 acids, respectively; *C. diphtheria*, one major homologue at m/z 495 for C32:0 corynomycolic acid $C_{32}H_{64}O_3$.

Fig. 5 depicts mass spectra profiles of mycolic acids (MAs) from nocardia. *N. brasillensis*, C54, C56, C58 with 2 and 3 double bonds; *N. asteroides*, C54:2, C56:2. The value m/z 828 corresponds to homologue $C_{56}H_{108}O_3$ with 2 double
25 bonds; *N. rubra*, C40 to C48 with 1 and 2 double bonds; *N. coralline*, C40 to 46 with 1 and 2 double bonds.

Fig. 6 depicts mass spectra profiles of mycolic acids (MAs) from various Mycobacterium tuberculosis (MTB) strains. The major MA species in *M. tuberculosis* are alpha and methoxy-MA, such as m/z 1,136 for alpha-MA
30 homologue $C_{78}H_{142}O_3$ and m/z 1,252 for methoxy-MA $C_{85}H_{168}O_4$; while *M. bovis*

BCG contains alpha-MA as well as Keto-MA, such as m/z 1,236 for keto-MA $C_{84}H_{164}O_4$.

Fig. 7 depicts mass spectra profiles of mycolic acids (MAs) of non tuberculous mycobacteria: absence of oxygenated MA; alpha MA only: *M. fallax*,
5 *M. triviae*, alpha and alpha' MA: *M. abscessus*, *M. chelonae*.

Fig. 8 depicts mass spectra profiles of mycolic acids (MAs) of non tuberculous mycobacteria: alpha, keto, methoxy: *M. gordonae*, *M. kansasii*, *M. ulcerans*.

Fig. 9 depicts mass spectra profiles of mycolic acids (MAs) of non
10 tuberculous mycobacteria: alpha, alpha', epoxy: *M. chitae*, *M. farcinogenes*, *M. fortuitum*, *M. porcinum*, *M. senegalense*, *M. smegmatis*.

Fig. 10 depicts mass spectra profiles of mycolic acids (MAs) of non tuberculous mycobacteria: alpha, keto, wax: *M. avium* complex (MAC), *M. phlei*,
15 *M. xenopi*. The presence of wax ester is evoked by the existence of dicarboxylic acids, at m/z 916 for the homologue $C_{60}H_{116}O_5$.

Fig. 11 depicts mass spectra profiles of mycolic acids (MAs) of non tuberculous mycobacteria: alpha, alpha', keto: *M. simiae*.

Fig. 12 depicts mass spectra profiles of mycolic acids (MAs) of non tuberculous mycobacteria: alpha, omega-1 methoxy: *M. alvei*.

20 Figs. 13A-B depict tandem mass spectrometry of mycolic acids (MAs). Corresponding ESI/MS and MS/MS results for MAs derived from *Nocadia asteroides* (Fig. 13A) and *Corynebacterium diphtheria* (Fig. 13B) are shown. Product ion analysis (MS/MS) of m/z 814 indicating the presence of C14:0 (m/z 227), C16:0 (m/z 255), and C18:0 (m/z 283) fatty acyls in the alpha-branch are
25 shown (Fig. 13A insert). Product ion analysis (MS/MS) of m/z 523 indicating the presence of C16:1 (m/z 253), C18:0 (m/z 283) fatty acyls in the alpha-branch are also shown (Fig. 13B insert).

Fig. 14 depicts a comparison of mycolic acid (MA) profiles of extracts from cultured mycobacterial strains with MA extracts from *Mycobacterium tuberculosis*
30 (MTB)-infected mice and mycobacteria-infected human patients. MA profiles were subject to hierarchical cluster analysis with Euclidean distance as the distance metric. All sputum samples from TB patients clearly grouped with the MA profiles derived

from MTB strains (box), possibly by the presence of abundant MA species with C26:0 fatty acyl alpha-branch in both alpha- and methoxy-MAs (arrow). The same was true for MA isolated from lungs of TB infected mice. The MA profile of NTM isolate-1, genetically characterized as *Mycobacterium avium* complex (MAC), aligned with MAC in the analysis. NTM sputum-2 and NTM isolate-2 (from the same patient) aligned closest with the MA profile of *M. chelonae*. Interestingly, strain classification of NTM-2 identified it as *M. massiliense*, which is indistinguishable from *M. chelonae*/*M. abscessus* by partial 16S rRNA gene sequencing.

DETAILED DESCRIPTION OF THE INVENTION

A description of example embodiments of the invention follows.

As described herein, Applicants have discovered that electrospray ionization mass spectrometry analysis of samples containing *Mycobacterium tuberculosis* yield particular profiles of mycolic acids (α -alkyl- β -hydroxy branched chain fatty acids), and that the presence of *Mycobacterium tuberculosis* can be assessed by the presentation of the profile of mycolic acids. Mycolic acids, α -alkyl- β -hydroxy branched chain fatty acids, are major envelope components of mycobacteria; they are cell wall-associated bacterial molecules and are not synthesized by the human body. Thus, the presence of mycolic acids is indicative of bacterial infection. Through electrospray ionization tandem mass spectrometry (ESI/MS), comprehensive lists of mycolic acid precursor ions and corresponding fragment ions were generated for multiple reaction monitoring (MRM). Quantification of mycolic acids, and in particular C26 α -mycolic acids, is now possible. The presence of particular mycolic acid profiles can therefore be used to diagnose active tuberculosis infection in an individual, as well as to assess the efficacy of treatment for tuberculosis in an individual.

The methods of the invention utilize a test sample. The term, "test sample," as used herein, refers to a sample that is suspected of, or confirmed as, containing *Mycobacterium tuberculosis*. Representative test samples include samples of isolated bacteria; bodily fluid samples (e.g., sputum); and tissue samples (e.g., lung

tissue). The sample can be obtained, for example, from an individual suspected of having an active tuberculosis infection, or, in the case of the methods for assessing efficacy of treatment of tuberculosis, from an individual known to have or previously diagnosed with an active tuberculosis infection. The HIV status of the individual is immaterial, as the methods are appropriate for individuals with HIV positive or HIV negative status. The individual can be a human being, and can also be another mammal (e.g., a mouse used as a model for *Mycobacterium tuberculosis* infection). If necessary or desired, a sample can be processed, such as to isolate bacteria therein, or to concentrate the sample, or to digest or decontaminate components of the sample.

In the methods of the invention, electrospray ionization mass spectrometry (ESI/MS), such as tandem ESI/MS, is used on a test sample. For example, mass spectrometry targeted analysis by multiple reaction monitoring (MRM) can be used. In MRM, a precursor ion of interest is selected in a first mass analyzer of a tandem mass spectrometer and fragmented in the collision cell, and a characteristic product ion (e.g., an acyl chain) is then selected in the second mass analyzer. Different mycolic acids can then be selectively monitored using transition pairs, using characteristic transitions specific for each species of mycolic acids as have been identified herein and described in the Supplementary Table 1 below. A set of characteristic transitions identifiable using these methods is referred to herein as a “profile” of the particular mycolic acids.

Supplementary Table 1

MRM Transitions	No. of Carbon	No. of Hydrogen	No. of Oxygen	α -branch Fatty acyls	class
1332.4/395.3	92	180	3	26:0	alpha
1318.4/395.3	91	178	3	26:0	alpha
1304.3/395.3	90	176	3	26:0	alpha
1290.3/395.3	89	174	3	26:0	alpha
1276.3/395.3	88	172	3	26:0	alpha
1262.3/395.3	87	170	3	26:0	alpha
1248.3/395.3	86	168	3	26:0	alpha
1234.3/395.3	85	166	3	26:0	alpha
1220.3/395.3	84	164	3	26:0	alpha
1206.2/395.3	83	162	3	26:0	alpha
1192.2/395.3	82	160	3	26:0	alpha
1178.2/395.3	81	158	3	26:0	alpha
1164.2/395.3	80	156	3	26:0	alpha
1150.2/395.3	79	154	3	26:0	alpha
1136.2/395.3	78	152	3	26:0	alpha
1122.1/395.3	77	150	3	26:0	alpha
1108.1/395.3	76	148	3	26:0	alpha
1094.1/395.3	75	146	3	26:0	alpha
1080.1/395.3	74	144	3	26:0	alpha
1066.1/395.3	73	142	3	26:0	alpha
1052.1/395.3	72	140	3	26:0	alpha
1038.1/395.3	71	138	3	26:0	alpha
1024.0/395.3	70	136	3	26:0	alpha
1010.0/395.3	69	134	3	26:0	alpha
996.0/395.3	68	132	3	26:0	alpha
982.0/395.3	67	130	3	26:0	alpha
968.0/395.3	66	128	3	26:0	alpha
954.0/395.3	65	126	3	26:0	alpha
939.9/395.3	64	124	3	26:0	alpha
925.9/395.3	63	122	3	26:0	alpha
911.9/395.3	62	120	3	26:0	alpha
897.9/395.3	61	118	3	26:0	alpha
883.9/395.3	60	116	3	26:0	alpha
869.9/395.3	59	114	3	26:0	alpha
855.9/395.3	58	112	3	26:0	alpha
1318.4/367.3	91	178	3	24:0	alpha
1304.3/367.3	90	176	3	24:0	alpha
1290.3/367.3	89	174	3	24:0	alpha
1276.3/367.3	88	172	3	24:0	alpha
1262.3/367.3	87	170	3	24:0	alpha
1248.3/367.3	86	168	3	24:0	alpha
1234.3/367.3	85	166	3	24:0	alpha
1220.3/367.3	84	164	3	24:0	alpha
1206.2/367.3	83	162	3	24:0	alpha
1192.2/367.3	82	160	3	24:0	alpha
1178.2/367.3	81	158	3	24:0	alpha
1164.2/367.3	80	156	3	24:0	alpha
1150.2/367.3	79	154	3	24:0	alpha
1136.2/367.3	78	152	3	24:0	alpha
1122.1/367.3	77	150	3	24:0	alpha
1108.1/367.3	76	148	3	24:0	alpha
1094.1/367.3	75	146	3	24:0	alpha
1080.1/367.3	74	144	3	24:0	alpha
1066.1/367.3	73	142	3	24:0	alpha
1052.1/367.3	72	140	3	24:0	alpha
1038.1/367.3	71	138	3	24:0	alpha
1024.0/367.3	70	136	3	24:0	alpha

1010.0/367.3	89	134	3	24:0	alpha
996.0/367.3	68	132	3	24:0	alpha
982.0/367.3	67	130	3	24:0	alpha
968.0/367.3	66	128	3	24:0	alpha
954.0/367.3	65	126	3	24:0	alpha
939.9/367.3	64	124	3	24:0	alpha
925.9/367.3	63	122	3	24:0	alpha
911.9/367.3	62	120	3	24:0	alpha
897.9/367.3	61	118	3	24:0	alpha
883.9/367.3	60	116	3	24:0	alpha
869.9/367.3	59	114	3	24:0	alpha
855.9/367.3	58	112	3	24:0	alpha
1318.4/339.2	91	178	3	22:0	alpha
1304.3/339.2	90	176	3	22:0	alpha
1290.3/339.2	89	174	3	22:0	alpha
1276.3/339.2	88	172	3	22:0	alpha
1262.3/339.2	87	170	3	22:0	alpha
1248.3/339.2	86	168	3	22:0	alpha
1234.3/339.2	85	166	3	22:0	alpha
1220.3/339.2	84	164	3	22:0	alpha
1206.2/339.2	83	162	3	22:0	alpha
1192.2/339.2	82	160	3	22:0	alpha
1178.2/339.2	81	158	3	22:0	alpha
1164.2/339.2	80	156	3	22:0	alpha
1150.2/339.2	79	154	3	22:0	alpha
1136.2/339.2	78	152	3	22:0	alpha
1122.1/339.2	77	150	3	22:0	alpha
1108.1/339.2	76	148	3	22:0	alpha
1094.1/339.2	75	146	3	22:0	alpha
1080.1/339.2	74	144	3	22:0	alpha
1066.1/339.2	73	142	3	22:0	alpha
1052.1/339.2	72	140	3	22:0	alpha
1038.1/339.2	71	138	3	22:0	alpha
1024.0/339.2	70	136	3	22:0	alpha
1010.0/339.2	69	134	3	22:0	alpha
996.0/339.2	68	132	3	22:0	alpha
982.0/339.2	67	130	3	22:0	alpha
968.0/339.2	66	128	3	22:0	alpha
954.0/339.2	65	126	3	22:0	alpha
939.9/339.2	64	124	3	22:0	alpha
925.9/339.2	63	122	3	22:0	alpha
911.9/339.2	62	120	3	22:0	alpha
897.9/339.2	61	118	3	22:0	alpha
883.9/339.2	60	116	3	22:0	alpha
869.9/339.2	59	114	3	22:0	alpha
855.9/339.2	58	112	3	22:0	alpha
1318.4/311.2	91	178	3	20:0	alpha
1304.3/311.2	90	176	3	20:0	alpha
1290.3/311.2	89	174	3	20:0	alpha
1276.3/311.2	88	172	3	20:0	alpha
1262.3/311.2	87	170	3	20:0	alpha
1248.3/311.2	86	168	3	20:0	alpha
1234.3/311.2	85	166	3	20:0	alpha
1220.3/311.2	84	164	3	20:0	alpha
1206.2/311.2	83	162	3	20:0	alpha
1192.2/311.2	82	160	3	20:0	alpha
1178.2/311.2	81	158	3	20:0	alpha
1164.2/311.2	80	156	3	20:0	alpha
1150.2/311.2	79	154	3	20:0	alpha
1136.2/311.2	78	152	3	20:0	alpha
1122.1/311.2	77	150	3	20:0	alpha
1108.1/311.2	76	148	3	20:0	alpha
1094.1/311.2	75	146	3	20:0	alpha
1080.1/311.2	74	144	3	20:0	alpha
1066.1/311.2	73	142	3	20:0	alpha
1052.1/311.2	72	140	3	20:0	alpha
1038.1/311.2	71	138	3	20:0	alpha
1024.0/311.2	70	136	3	20:0	alpha
1010.0/311.2	69	134	3	20:0	alpha
996.0/311.2	68	132	3	20:0	alpha
982.0/311.2	67	130	3	20:0	alpha
968.0/311.2	66	128	3	20:0	alpha

954.0/311.2	65	126	3	20:0	alpha
939.9/311.2	64	124	3	20:0	alpha
925.9/311.2	63	122	3	20:0	alpha
911.9/311.2	62	120	3	20:0	alpha
897.9/311.2	61	118	3	20:0	alpha
883.9/311.2	60	116	3	20:0	alpha
869.9/311.2	59	114	3	20:0	alpha
855.9/311.2	58	112	3	20:0	alpha
841.8/311.2	57	110	3	20:0	alpha
827.8/311.2	56	108	3	20:0	alpha
813.8/311.2	55	106	3	20:0	alpha
799.8/311.2	54	104	3	20:0	alpha
785.8/311.2	53	102	3	20:0	alpha
771.8/311.2	52	100	3	20:0	alpha
1152.2/395.3	79	156	3	26:0	alpha prime
1138.2/395.3	78	154	3	26:0	alpha prime
1124.2/395.3	77	152	3	26:0	alpha prime
1110.1/395.3	76	150	3	26:0	alpha prime
1096.1/395.3	75	148	3	26:0	alpha prime
1082.1/395.3	74	146	3	26:0	alpha prime
1068.1/395.3	73	144	3	26:0	alpha prime
1054.1/395.3	72	142	3	26:0	alpha prime
1040.1/395.3	71	140	3	26:0	alpha prime
1026.1/395.3	70	138	3	26:0	alpha prime
1012.0/395.3	69	136	3	26:0	alpha prime
998.0/395.3	68	134	3	26:0	alpha prime
984.0/395.3	67	132	3	26:0	alpha prime
970.0/395.3	66	130	3	26:0	alpha prime
956.0/395.3	65	128	3	26:0	alpha prime
942.0/395.3	64	126	3	26:0	alpha prime
927.9/395.3	63	124	3	26:0	alpha prime
913.9/395.3	62	122	3	26:0	alpha prime
899.9/395.3	61	120	3	26:0	alpha prime
885.9/395.3	60	118	3	26:0	alpha prime
871.9/395.3	59	116	3	26:0	alpha prime
857.9/395.3	58	114	3	26:0	alpha prime
843.9/395.3	57	112	3	26:0	alpha prime
829.8/395.3	56	110	3	26:0	alpha prime
815.8/395.3	55	108	3	26:0	alpha prime
1110.1/367.3	76	150	3	24:0	alpha prime
1096.1/367.3	75	148	3	24:0	alpha prime
1082.1/367.3	74	146	3	24:0	alpha prime
1068.1/367.3	73	144	3	24:0	alpha prime
1054.1/367.3	72	142	3	24:0	alpha prime
1040.1/367.3	71	140	3	24:0	alpha prime
1026.1/367.3	70	138	3	24:0	alpha prime
1012.0/367.3	69	136	3	24:0	alpha prime
998.0/367.3	68	134	3	24:0	alpha prime
984.0/367.3	67	132	3	24:0	alpha prime
970.0/367.3	66	130	3	24:0	alpha prime
956.0/367.3	65	128	3	24:0	alpha prime
942.0/367.3	64	126	3	24:0	alpha prime
927.9/367.3	63	124	3	24:0	alpha prime
913.9/367.3	62	122	3	24:0	alpha prime
899.9/367.3	61	120	3	24:0	alpha prime
885.9/367.3	60	118	3	24:0	alpha prime
871.9/367.3	59	116	3	24:0	alpha prime
857.9/367.3	58	114	3	24:0	alpha prime
843.9/367.3	57	112	3	24:0	alpha prime
829.8/367.3	56	110	3	24:0	alpha prime
815.8/367.3	55	108	3	24:0	alpha prime
1110.1/339.2	76	160	3	22:0	alpha prime
1096.1/339.2	75	148	3	22:0	alpha prime
1082.1/339.2	74	146	3	22:0	alpha prime
1068.1/339.2	73	144	3	22:0	alpha prime
1054.1/339.2	72	142	3	22:0	alpha prime
1040.1/339.2	71	140	3	22:0	alpha prime
1026.1/339.2	70	138	3	22:0	alpha prime
1012.0/339.2	69	136	3	22:0	alpha prime
998.0/339.2	68	134	3	22:0	alpha prime
984.0/339.2	67	132	3	22:0	alpha prime
970.0/339.2	66	130	3	22:0	alpha prime

956.0/339.2	65	128	3	22:0	alpha prime
942.0/339.2	64	126	3	22:0	alpha prime
927.9/339.2	63	124	3	22:0	alpha prime
913.9/339.2	62	122	3	22:0	alpha prime
899.9/339.2	61	120	3	22:0	alpha prime
885.9/339.2	60	118	3	22:0	alpha prime
871.9/339.2	59	116	3	22:0	alpha prime
857.9/339.2	58	114	3	22:0	alpha prime
843.9/339.2	57	112	3	22:0	alpha prime
829.8/339.2	56	110	3	22:0	alpha prime
815.8/339.2	55	108	3	22:0	alpha prime
1110.1/311.2	76	150	3	20:0	alpha prime
1096.1/311.2	75	148	3	20:0	alpha prime
1082.1/311.2	74	146	3	20:0	alpha prime
1068.1/311.2	73	144	3	20:0	alpha prime
1054.1/311.2	72	142	3	20:0	alpha prime
1040.1/311.2	71	140	3	20:0	alpha prime
1026.1/311.2	70	138	3	20:0	alpha prime
1012.0/311.2	69	136	3	20:0	alpha prime
998.0/311.2	68	134	3	20:0	alpha prime
984.0/311.2	67	132	3	20:0	alpha prime
970.0/311.2	66	130	3	20:0	alpha prime
956.0/311.2	65	128	3	20:0	alpha prime
942.0/311.2	64	126	3	20:0	alpha prime
927.9/311.2	63	124	3	20:0	alpha prime
913.9/311.2	62	122	3	20:0	alpha prime
899.9/311.2	61	120	3	20:0	alpha prime
885.9/311.2	60	118	3	20:0	alpha prime
871.9/311.2	59	116	3	20:0	alpha prime
857.9/311.2	58	114	3	20:0	alpha prime
843.9/311.2	57	112	3	20:0	alpha prime
829.8/311.2	56	110	3	20:0	alpha prime
815.8/311.2	55	108	3	20:0	alpha prime
1232.3/395.3	85	164	3	26:0	alpha - 2
1204.2/395.3	83	160	3	26:0	alpha - 2
1190.2/395.3	82	158	3	26:0	alpha - 2
1176.2/395.3	81	156	3	26:0	alpha - 2
1162.2/395.3	80	154	3	26:0	alpha - 2
1148.2/395.3	79	152	3	26:0	alpha - 2
1134.1/395.3	78	150	3	26:0	alpha - 2
1120.1/395.3	77	148	3	26:0	alpha - 2
1106.1/395.3	76	146	3	26:0	alpha - 2
1092.1/395.3	75	144	3	26:0	alpha - 2
1078.1/395.3	74	142	3	26:0	alpha - 2
811.8/395.3	55	104	3	26:0	alpha - 2
797.8/395.3	54	102	3	26:0	alpha - 2
783.8/395.3	53	100	3	26:0	alpha - 2
769.7/395.3	52	98	3	26:0	alpha - 2
755.7/395.3	51	96	3	26:0	alpha - 2
741.7/395.3	50	94	3	26:0	alpha - 2
727.7/395.3	49	92	3	26:0	alpha - 2
713.7/395.3	48	90	3	26:0	alpha - 2
699.7/395.3	47	88	3	26:0	alpha - 2
1232.3/367.3	85	164	3	24:0	alpha - 2
1204.2/367.3	83	160	3	24:0	alpha - 2
1190.2/367.3	82	158	3	24:0	alpha - 2
1176.2/367.3	81	156	3	24:0	alpha - 2
1162.2/367.3	80	154	3	24:0	alpha - 2
1148.2/367.3	79	152	3	24:0	alpha - 2
1134.1/367.3	78	150	3	24:0	alpha - 2
1120.1/367.3	77	148	3	24:0	alpha - 2
1106.1/367.3	76	146	3	24:0	alpha - 2
1092.1/367.3	75	144	3	24:0	alpha - 2
1078.1/367.3	74	142	3	24:0	alpha - 2
811.8/367.3	55	104	3	24:0	alpha - 2
797.8/367.3	54	102	3	24:0	alpha - 2
783.8/367.3	53	100	3	24:0	alpha - 2
769.7/367.3	52	98	3	24:0	alpha - 2
755.7/367.3	51	96	3	24:0	alpha - 2
741.7/367.3	50	94	3	24:0	alpha - 2
727.7/367.3	49	92	3	24:0	alpha - 2
713.7/367.3	48	90	3	24:0	alpha - 2

699.7/367.3	47	88	3	24:0	alpha - 2
1232.3/339.2	85	164	3	22:0	alpha - 2
1204.2/339.2	83	160	3	22:0	alpha - 2
1190.2/339.2	82	158	3	22:0	alpha - 2
1176.2/339.2	81	156	3	22:0	alpha - 2
1162.2/339.2	80	154	3	22:0	alpha - 2
1148.2/339.2	79	152	3	22:0	alpha - 2
1134.1/339.2	78	150	3	22:0	alpha - 2
1120.1/339.2	77	148	3	22:0	alpha - 2
1106.1/339.2	76	146	3	22:0	alpha - 2
1092.1/339.2	75	144	3	22:0	alpha - 2
1078.1/339.2	74	142	3	22:0	alpha - 2
811.8/339.2	55	104	3	22:0	alpha - 2
797.8/339.2	54	102	3	22:0	alpha - 2
783.8/339.2	53	100	3	22:0	alpha - 2
769.7/339.2	52	98	3	22:0	alpha - 2
755.7/339.2	51	96	3	22:0	alpha - 2
741.7/339.2	50	94	3	22:0	alpha - 2
727.7/339.2	49	92	3	22:0	alpha - 2
713.7/339.2	48	90	3	22:0	alpha - 2
699.7/339.2	47	88	3	22:0	alpha - 2
1232.3/311.2	85	164	3	20:0	alpha - 2
1204.2/311.2	83	160	3	20:0	alpha - 2
1190.2/311.2	82	158	3	20:0	alpha - 2
1176.2/311.2	81	156	3	20:0	alpha - 2
1162.2/311.2	80	154	3	20:0	alpha - 2
1148.2/311.2	79	152	3	20:0	alpha - 2
1134.1/311.2	78	150	3	20:0	alpha - 2
1120.1/311.2	77	148	3	20:0	alpha - 2
1106.1/311.2	76	146	3	20:0	alpha - 2
1092.1/311.2	75	144	3	20:0	alpha - 2
1078.1/311.2	74	142	3	20:0	alpha - 2
811.8/311.2	55	104	3	20:0	alpha - 2
797.8/311.2	54	102	3	20:0	alpha - 2
783.8/311.2	53	100	3	20:0	alpha - 2
769.7/311.2	52	98	3	20:0	alpha - 2
755.7/311.2	51	96	3	20:0	alpha - 2
741.7/311.2	50	94	3	20:0	alpha - 2
727.7/311.2	49	92	3	20:0	alpha - 2
713.7/311.2	48	90	3	20:0	alpha - 2
699.7/311.2	47	88	3	20:0	alpha - 2
1362.4/395.3	93	182	4	26:0	keto/epoxy/ ω -1
1348.4/395.3	92	180	4	26:0	keto/epoxy/ ω -1
1334.4/395.3	91	178	4	26:0	keto/epoxy/ ω -1
1320.3/395.3	90	176	4	26:0	keto/epoxy/ ω -1
1306.3/395.3	89	174	4	26:0	keto/epoxy/ ω -1
1292.3/395.3	88	172	4	26:0	keto/epoxy/ ω -1
1278.3/395.3	87	170	4	26:0	keto/epoxy/ ω -1
1264.3/395.3	86	168	4	26:0	keto/epoxy/ ω -1
1250.3/395.3	85	166	4	26:0	keto/epoxy/ ω -1
1236.3/395.3	84	164	4	26:0	keto/epoxy/ ω -1
1222.2/395.3	83	162	4	26:0	keto/epoxy/ ω -1
1208.2/395.3	82	160	4	26:0	keto/epoxy/ ω -1
1194.2/395.3	81	158	4	26:0	keto/epoxy/ ω -1
1180.2/395.3	80	156	4	26:0	keto/epoxy/ ω -1
1166.2/395.3	79	154	4	26:0	keto/epoxy/ ω -1
1152.2/395.3	78	152	4	26:0	keto/epoxy/ ω -1
1138.1/395.3	77	150	4	26:0	keto/epoxy/ ω -1
1124.1/395.3	76	148	4	26:0	keto/epoxy/ ω -1
1110.1/395.3	75	146	4	26:0	keto/epoxy/ ω -1
1096.1/395.3	74	144	4	26:0	keto/epoxy/ ω -1
1082.1/395.3	73	142	4	26:0	keto/epoxy/ ω -1
1068.1/395.3	72	140	4	26:0	keto/epoxy/ ω -1
1054.0/395.3	71	138	4	26:0	keto/epoxy/ ω -1
1040.0/395.3	70	136	4	26:0	keto/epoxy/ ω -1
1026.0/395.3	69	134	4	26:0	keto/epoxy/ ω -1
1012.0/395.3	68	132	4	26:0	keto/epoxy/ ω -1
998.0/395.3	67	130	4	26:0	keto/epoxy/ ω -1
984.0/395.3	66	128	4	26:0	keto/epoxy/ ω -1
970.0/395.3	65	126	4	26:0	keto/epoxy/ ω -1
955.9/395.3	64	124	4	26:0	keto/epoxy/ ω -1
941.9/395.3	63	122	4	26:0	keto/epoxy/ ω -1

927.9/395.3	62	120	4	26:0	keto/epoxy/w-1
913.9/395.3	61	118	4	26:0	keto/epoxy/w-1
899.9/395.3	60	116	4	26:0	keto/epoxy/w-1
885.9/395.3	59	114	4	26:0	keto/epoxy/w-1
871.8/395.3	58	112	4	26:0	keto/epoxy/w-1
1362.4/367.3	93	182	4	24:0	keto/epoxy/w-1
1348.4/367.3	92	180	4	24:0	keto/epoxy/w-1
1334.4/367.3	91	178	4	24:0	keto/epoxy/w-1
1320.3/367.3	90	176	4	24:0	keto/epoxy/w-1
1306.3/367.3	89	174	4	24:0	keto/epoxy/w-1
1292.3/367.3	88	172	4	24:0	keto/epoxy/w-1
1278.3/367.3	87	170	4	24:0	keto/epoxy/w-1
1264.3/367.3	86	168	4	24:0	keto/epoxy/w-1
1250.3/367.3	85	166	4	24:0	keto/epoxy/w-1
1236.3/367.3	84	164	4	24:0	keto/epoxy/w-1
1222.2/367.3	83	162	4	24:0	keto/epoxy/w-1
1208.2/367.3	82	160	4	24:0	keto/epoxy/w-1
1194.2/367.3	81	158	4	24:0	keto/epoxy/w-1
1180.2/367.3	80	156	4	24:0	keto/epoxy/w-1
1166.2/367.3	79	154	4	24:0	keto/epoxy/w-1
1152.2/367.3	78	152	4	24:0	keto/epoxy/w-1
1138.1/367.3	77	150	4	24:0	keto/epoxy/w-1
1124.1/367.3	76	148	4	24:0	keto/epoxy/w-1
1110.1/367.3	75	146	4	24:0	keto/epoxy/w-1
1096.1/367.3	74	144	4	24:0	keto/epoxy/w-1
1082.1/367.3	73	142	4	24:0	keto/epoxy/w-1
1068.1/367.3	72	140	4	24:0	keto/epoxy/w-1
1054.0/367.3	71	138	4	24:0	keto/epoxy/w-1
1040.0/367.3	70	136	4	24:0	keto/epoxy/w-1
1026.0/367.3	69	134	4	24:0	keto/epoxy/w-1
1012.0/367.3	68	132	4	24:0	keto/epoxy/w-1
998.0/367.3	67	130	4	24:0	keto/epoxy/w-1
984.0/367.3	66	128	4	24:0	keto/epoxy/w-1
970.0/367.3	65	126	4	24:0	keto/epoxy/w-1
955.9/367.3	64	124	4	24:0	keto/epoxy/w-1
941.9/367.3	63	122	4	24:0	keto/epoxy/w-1
927.9/367.3	62	120	4	24:0	keto/epoxy/w-1
913.9/367.3	61	118	4	24:0	keto/epoxy/w-1
899.9/367.3	60	116	4	24:0	keto/epoxy/w-1
885.9/367.3	59	114	4	24:0	keto/epoxy/w-1
871.8/367.3	58	112	4	24:0	keto/epoxy/w-1
1362.4/339.3	93	182	4	22:0	keto/epoxy/w-1
1348.4/339.2	92	180	4	22:0	keto/epoxy/w-1
1334.4/339.2	91	178	4	22:0	keto/epoxy/w-1
1320.3/339.2	90	176	4	22:0	keto/epoxy/w-1
1306.3/339.2	89	174	4	22:0	keto/epoxy/w-1
1292.3/339.2	88	172	4	22:0	keto/epoxy/w-1
1278.3/339.2	87	170	4	22:0	keto/epoxy/w-1
1264.3/339.2	86	168	4	22:0	keto/epoxy/w-1
1250.3/339.2	85	166	4	22:0	keto/epoxy/w-1
1236.3/339.2	84	164	4	22:0	keto/epoxy/w-1
1222.2/339.2	83	162	4	22:0	keto/epoxy/w-1
1208.2/339.2	82	160	4	22:0	keto/epoxy/w-1
1194.2/339.2	81	158	4	22:0	keto/epoxy/w-1
1180.2/339.2	80	156	4	22:0	keto/epoxy/w-1
1166.2/339.2	79	154	4	22:0	keto/epoxy/w-1
1152.2/339.2	78	152	4	22:0	keto/epoxy/w-1
1138.1/339.2	77	150	4	22:0	keto/epoxy/w-1
1124.1/339.2	76	148	4	22:0	keto/epoxy/w-1
1110.1/339.2	75	146	4	22:0	keto/epoxy/w-1
1096.1/339.2	74	144	4	22:0	keto/epoxy/w-1
1082.1/339.2	73	142	4	22:0	keto/epoxy/w-1
1068.1/339.2	72	140	4	22:0	keto/epoxy/w-1
1054.0/339.2	71	138	4	22:0	keto/epoxy/w-1
1040.0/339.2	70	136	4	22:0	keto/epoxy/w-1
1026.0/339.2	69	134	4	22:0	keto/epoxy/w-1
1012.0/339.2	68	132	4	22:0	keto/epoxy/w-1
998.0/339.2	67	130	4	22:0	keto/epoxy/w-1
984.0/339.2	66	128	4	22:0	keto/epoxy/w-1
970.0/339.2	65	126	4	22:0	keto/epoxy/w-1
955.9/339.2	64	124	4	22:0	keto/epoxy/w-1
941.9/339.2	63	122	4	22:0	keto/epoxy/w-1

927.9/339.2	62	120	4	22:0	keto/epoxy/ ω -1
913.9/339.2	61	118	4	22:0	keto/epoxy/ ω -1
899.9/339.2	60	116	4	22:0	keto/epoxy/ ω -1
885.9/339.2	59	114	4	22:0	keto/epoxy/ ω -1
871.8/339.2	58	112	4	22:0	keto/epoxy/ ω -1
1362.4/311.2	93	182	4	20:0	keto/epoxy/ ω -1
1348.4/311.2	92	180	4	20:0	keto/epoxy/ ω -1
1334.4/311.2	91	178	4	20:0	keto/epoxy/ ω -1
1320.3/311.2	90	176	4	20:0	keto/epoxy/ ω -1
1306.3/311.2	89	174	4	20:0	keto/epoxy/ ω -1
1292.3/311.2	88	172	4	20:0	keto/epoxy/ ω -1
1278.3/311.2	87	170	4	20:0	keto/epoxy/ ω -1
1264.3/311.2	86	168	4	20:0	keto/epoxy/ ω -1
1250.3/311.2	85	166	4	20:0	keto/epoxy/ ω -1
1236.3/311.2	84	164	4	20:0	keto/epoxy/ ω -1
1222.2/311.2	83	162	4	20:0	keto/epoxy/ ω -1
1208.2/311.2	82	160	4	20:0	keto/epoxy/ ω -1
1194.2/311.2	81	158	4	20:0	keto/epoxy/ ω -1
1180.2/311.2	80	156	4	20:0	keto/epoxy/ ω -1
1166.2/311.2	79	154	4	20:0	keto/epoxy/ ω -1
1152.2/311.2	78	152	4	20:0	keto/epoxy/ ω -1
1138.1/311.2	77	150	4	20:0	keto/epoxy/ ω -1
1124.1/311.2	76	148	4	20:0	keto/epoxy/ ω -1
1110.1/311.2	75	146	4	20:0	keto/epoxy/ ω -1
1096.1/311.2	74	144	4	20:0	keto/epoxy/ ω -1
1082.1/311.2	73	142	4	20:0	keto/epoxy/ ω -1
1068.1/311.2	72	140	4	20:0	keto/epoxy/ ω -1
1054.0/311.2	71	138	4	20:0	keto/epoxy/ ω -1
1040.0/311.2	70	136	4	20:0	keto/epoxy/ ω -1
1028.0/311.2	69	134	4	20:0	keto/epoxy/ ω -1
1012.0/311.2	68	132	4	20:0	keto/epoxy/ ω -1
998.0/311.2	67	130	4	20:0	keto/epoxy/ ω -1
984.0/311.2	66	128	4	20:0	keto/epoxy/ ω -1
970.0/311.2	65	126	4	20:0	keto/epoxy/ ω -1
956.9/311.2	64	124	4	20:0	keto/epoxy/ ω -1
941.9/311.2	63	122	4	20:0	keto/epoxy/ ω -1
927.9/311.2	62	120	4	20:0	keto/epoxy/ ω -1
913.9/311.2	61	118	4	20:0	keto/epoxy/ ω -1
899.9/311.2	60	116	4	20:0	keto/epoxy/ ω -1
885.9/311.2	59	114	4	20:0	keto/epoxy/ ω -1
871.8/311.2	58	112	4	20:0	keto/epoxy/ ω -1
1350.4/395.3	92	182	4	26:0	methoxy
1336.4/395.3	91	180	4	26:0	methoxy
1322.4/395.3	90	178	4	26:0	methoxy
1308.3/395.3	89	176	4	26:0	methoxy
1294.3/395.3	88	174	4	26:0	methoxy
1280.3/395.3	87	172	4	26:0	methoxy
1266.3/395.3	86	170	4	26:0	methoxy
1252.3/395.3	85	168	4	26:0	methoxy
1238.3/395.3	84	166	4	26:0	methoxy
1224.3/395.3	83	164	4	26:0	methoxy
1210.2/395.3	82	162	4	26:0	methoxy
1196.2/395.3	81	160	4	26:0	methoxy
1182.2/395.3	80	158	4	26:0	methoxy
1168.2/395.3	79	156	4	26:0	methoxy
1154.2/395.3	78	154	4	26:0	methoxy
1140.2/395.3	77	152	4	26:0	methoxy
1126.1/395.3	76	150	4	26:0	methoxy
1112.1/395.3	75	148	4	26:0	methoxy
1098.1/395.3	74	146	4	26:0	methoxy
1084.1/395.3	73	144	4	26:0	methoxy
1070.1/395.3	72	142	4	26:0	methoxy
1056.1/395.3	71	140	4	26:0	methoxy
1042.0/395.3	70	138	4	26:0	methoxy
1028.0/395.3	69	136	4	26:0	methoxy
1014.0/395.3	68	134	4	26:0	methoxy
1000.0/395.3	67	132	4	26:0	methoxy
986.0/395.3	66	130	4	26:0	methoxy
972.0/395.3	65	128	4	26:0	methoxy
958.0/395.3	64	126	4	26:0	methoxy
943.9/395.3	63	124	4	26:0	methoxy
929.9/395.3	62	122	4	26:0	methoxy

1350.4/367.3	92	182	4	24:0	methoxy
1336.4/367.3	91	180	4	24:0	methoxy
1322.4/367.3	90	178	4	24:0	methoxy
1308.3/367.3	89	176	4	24:0	methoxy
1294.3/367.3	88	174	4	24:0	methoxy
1280.3/367.3	87	172	4	24:0	methoxy
1266.3/367.3	86	170	4	24:0	methoxy
1252.3/367.3	85	168	4	24:0	methoxy
1238.3/367.3	84	166	4	24:0	methoxy
1224.3/367.3	83	164	4	24:0	methoxy
1210.2/367.3	82	162	4	24:0	methoxy
1196.2/367.3	81	160	4	24:0	methoxy
1182.2/367.3	80	158	4	24:0	methoxy
1168.2/367.3	79	156	4	24:0	methoxy
1154.2/367.3	78	154	4	24:0	methoxy
1140.2/367.3	77	152	4	24:0	methoxy
1126.1/367.3	76	150	4	24:0	methoxy
1112.1/367.3	75	148	4	24:0	methoxy
1098.1/367.3	74	146	4	24:0	methoxy
1084.1/367.3	73	144	4	24:0	methoxy
1070.1/367.3	72	142	4	24:0	methoxy
1056.1/367.3	71	140	4	24:0	methoxy
1042.0/367.3	70	138	4	24:0	methoxy
1028.0/367.3	69	136	4	24:0	methoxy
1014.0/367.3	68	134	4	24:0	methoxy
1000.0/367.3	67	132	4	24:0	methoxy
986.0/367.3	66	130	4	24:0	methoxy
972.0/367.3	65	128	4	24:0	methoxy
958.0/367.3	64	126	4	24:0	methoxy
943.9/367.3	63	124	4	24:0	methoxy
929.9/367.3	62	122	4	24:0	methoxy
1350.4/339.2	92	182	4	22:0	methoxy
1336.4/339.2	91	180	4	22:0	methoxy
1322.4/339.2	90	178	4	22:0	methoxy
1308.3/339.2	89	176	4	22:0	methoxy
1294.3/339.2	88	174	4	22:0	methoxy
1280.3/339.2	87	172	4	22:0	methoxy
1266.3/339.2	86	170	4	22:0	methoxy
1252.3/339.2	85	168	4	22:0	methoxy
1238.3/339.2	84	166	4	22:0	methoxy
1224.3/339.2	83	164	4	22:0	methoxy
1210.2/339.2	82	162	4	22:0	methoxy
1196.2/339.2	81	160	4	22:0	methoxy
1182.2/339.2	80	158	4	22:0	methoxy
1168.2/339.2	79	156	4	22:0	methoxy
1154.2/339.2	78	154	4	22:0	methoxy
1140.2/339.2	77	152	4	22:0	methoxy
1126.1/339.2	76	150	4	22:0	methoxy
1112.1/339.2	75	148	4	22:0	methoxy
1098.1/339.2	74	146	4	22:0	methoxy
1084.1/339.2	73	144	4	22:0	methoxy
1070.1/339.2	72	142	4	22:0	methoxy
1056.1/339.2	71	140	4	22:0	methoxy
1042.0/339.2	70	138	4	22:0	methoxy
1028.0/339.2	69	136	4	22:0	methoxy
1014.0/339.2	68	134	4	22:0	methoxy
1000.0/339.2	67	132	4	22:0	methoxy
986.0/339.2	66	130	4	22:0	methoxy
972.0/339.2	65	128	4	22:0	methoxy
958.0/339.2	64	126	4	22:0	methoxy
943.9/339.2	63	124	4	22:0	methoxy
929.9/339.2	62	122	4	22:0	methoxy
1350.4/311.2	92	182	4	20:0	methoxy
1336.4/311.2	91	180	4	20:0	methoxy
1322.4/311.2	90	178	4	20:0	methoxy
1308.3/311.2	89	176	4	20:0	methoxy
1294.3/311.2	88	174	4	20:0	methoxy
1280.3/311.2	87	172	4	20:0	methoxy
1266.3/311.2	86	170	4	20:0	methoxy
1252.3/311.2	85	168	4	20:0	methoxy
1238.3/311.2	84	166	4	20:0	methoxy
1224.3/311.2	83	164	4	20:0	methoxy

1210.2/311.2	82	162	4	20:0	methoxy
1196.2/311.2	81	160	4	20:0	methoxy
1182.2/311.2	80	158	4	20:0	methoxy
1168.2/311.2	79	156	4	20:0	methoxy
1154.2/311.2	78	154	4	20:0	methoxy
1140.2/311.2	77	152	4	20:0	methoxy
1126.1/311.2	76	150	4	20:0	methoxy
1112.1/311.1	75	148	4	20:0	methoxy
1098.1/311.2	74	146	4	20:0	methoxy
1084.1/311.2	73	144	4	20:0	methoxy
1070.1/311.2	72	142	4	20:0	methoxy
1056.1/311.2	71	140	4	20:0	methoxy
1042.0/311.2	70	138	4	20:0	methoxy
1028.0/311.2	69	136	4	20:0	methoxy
1014.0/311.2	68	134	4	20:0	methoxy
1000.0/311.2	67	132	4	20:0	methoxy
986.0/311.2	66	130	4	20:0	methoxy
972.0/311.2	65	128	4	20:0	methoxy
958.0/311.2	64	126	4	20:0	methoxy
943.9/311.2	63	124	4	20:0	methoxy
929.9/311.2	62	122	4	20:0	methoxy
1098.1/395.3	73	142	5	26:0	dicarboxy
1084.1/395.3	72	140	5	26:0	dicarboxy
1070.0/395.3	71	138	5	26:0	dicarboxy
1056.0/395.3	70	136	5	26:0	dicarboxy
1042.0/395.3	69	134	5	26:0	dicarboxy
1028.0/395.3	68	132	5	26:0	dicarboxy
1014.0/395.3	67	130	5	26:0	dicarboxy
1000.0/395.3	66	128	5	26:0	dicarboxy
985.9/395.3	65	126	5	26:0	dicarboxy
971.9/395.3	64	124	5	26:0	dicarboxy
957.9/395.3	63	122	5	26:0	dicarboxy
943.9/395.3	62	120	5	26:0	dicarboxy
929.9/395.3	61	118	5	26:0	dicarboxy
915.9/395.3	60	116	5	26:0	dicarboxy
901.9/395.3	59	114	5	26:0	dicarboxy
887.8/395.3	58	112	5	26:0	dicarboxy
873.8/395.3	57	110	5	26:0	dicarboxy
859.8/395.3	56	108	5	26:0	dicarboxy
845.8/395.3	55	106	5	26:0	dicarboxy
831.8/395.3	54	104	5	26:0	dicarboxy
817.6/395.3	53	102	5	26:0	dicarboxy
803.7/395.3	52	100	5	26:0	dicarboxy
1098.1/367.3	73	142	5	24:0	dicarboxy
1084.1/367.3	72	140	5	24:0	dicarboxy
1070.0/367.3	71	138	5	24:0	dicarboxy
1056.0/367.3	70	136	5	24:0	dicarboxy
1042.0/367.3	69	134	5	24:0	dicarboxy
1028.0/367.3	68	132	5	24:0	dicarboxy
1014.0/367.3	67	130	5	24:0	dicarboxy
1000.0/367.3	66	128	5	24:0	dicarboxy
985.9/367.3	65	126	5	24:0	dicarboxy
971.9/367.3	64	124	5	24:0	dicarboxy
957.9/367.3	63	122	5	24:0	dicarboxy
943.9/367.3	62	120	5	24:0	dicarboxy
929.9/367.3	61	118	5	24:0	dicarboxy
915.9/367.3	60	116	5	24:0	dicarboxy
901.9/367.3	59	114	5	24:0	dicarboxy
887.8/367.3	58	112	5	24:0	dicarboxy
873.8/367.3	57	110	5	24:0	dicarboxy
859.8/367.3	56	108	5	24:0	dicarboxy
845.8/367.3	55	106	5	24:0	dicarboxy
831.8/367.3	54	104	5	24:0	dicarboxy
817.8/367.3	53	102	5	24:0	dicarboxy
803.7/367.3	52	100	5	24:0	dicarboxy
1098.1/339.2	73	142	5	22:0	dicarboxy
1084.1/339.2	72	140	5	22:0	dicarboxy
1070.0/339.2	71	138	5	22:0	dicarboxy
1056.0/339.2	70	136	5	22:0	dicarboxy
1042.0/339.2	69	134	5	22:0	dicarboxy
1028.0/339.2	68	132	5	22:0	dicarboxy
1014.0/339.2	67	130	5	22:0	dicarboxy

1000.0/339.2	66	128	5	22:0	dicarboxy
985.9/339.2	65	126	5	22:0	dicarboxy
971.9/339.2	64	124	5	22:0	dicarboxy
957.9/339.2	63	122	5	22:0	dicarboxy
943.9/339.2	62	120	5	22:0	dicarboxy
929.9/339.2	61	118	5	22:0	dicarboxy
915.9/339.2	60	116	5	22:0	dicarboxy
901.9/339.2	59	114	5	22:0	dicarboxy
887.8/339.2	58	112	5	22:0	dicarboxy
873.8/339.2	57	110	5	22:0	dicarboxy
859.8/339.2	56	108	5	22:0	dicarboxy
845.8/339.2	55	106	5	22:0	dicarboxy
831.8/339.2	54	104	5	22:0	dicarboxy
817.8/339.2	53	102	5	22:0	dicarboxy
803.7/339.2	52	100	5	22:0	dicarboxy
1098.1/311.2	73	142	5	20:0	dicarboxy
1084.1/311.2	72	140	5	20:0	dicarboxy
1070.0/311.2	71	138	5	20:0	dicarboxy
1056.0/311.2	70	136	5	20:0	dicarboxy
1042.0/311.2	69	134	5	20:0	dicarboxy
1028.0/311.2	68	132	5	20:0	dicarboxy
1014.0/311.2	67	130	5	20:0	dicarboxy
1000.0/311.2	66	128	5	20:0	dicarboxy
985.9/311.2	65	126	5	20:0	dicarboxy
971.9/311.2	64	124	5	20:0	dicarboxy
957.9/311.2	63	122	5	20:0	dicarboxy
943.9/311.2	62	120	5	20:0	dicarboxy
929.9/311.2	61	118	5	20:0	dicarboxy
915.9/311.2	60	116	5	20:0	dicarboxy
901.9/311.2	59	114	5	20:0	dicarboxy
887.8/311.2	58	112	5	20:0	dicarboxy
873.8/311.2	57	110	5	20:0	dicarboxy
859.8/311.2	56	108	5	20:0	dicarboxy
845.8/311.2	55	106	5	20:0	dicarboxy
831.8/311.2	54	104	5	20:0	dicarboxy
817.8/311.2	53	102	5	20:0	dicarboxy
803.7/311.2	52	100	5	20:0	dicarboxy
909.9/297.2	62	118	3	19:0	alpha
883.9/297.2	60	116	3	19:0	alpha
881.9/297.2	60	114	3	19:0	alpha
871.9/297.2	59	116	3	19:0	alpha
869.9/297.2	59	114	3	19:0	alpha
867.9/297.2	59	112	3	19:0	alpha
855.9/297.2	58	112	3	19:0	alpha
853.8/297.2	58	110	3	19:0	alpha
843.9/297.2	57	112	3	19:0	alpha
841.8/297.2	57	110	3	19:0	alpha
839.8/297.2	57	108	3	19:0	alpha
827.8/297.2	56	108	3	19:0	alpha
825.8/297.2	56	106	3	19:0	alpha
815.8/297.2	55	108	3	19:0	alpha
813.8/297.2	55	106	3	19:0	alpha
811.8/297.2	55	104	3	19:0	alpha
801.8/297.2	54	106	3	19:0	alpha
799.8/297.2	54	104	3	19:0	alpha
797.8/297.2	54	102	3	19:0	alpha
787.8/297.2	53	104	3	19:0	alpha
785.8/297.2	53	102	3	19:0	alpha
783.8/297.2	53	100	3	19:0	alpha
773.8/297.2	52	102	3	19:0	alpha
771.8/297.2	52	100	3	19:0	alpha
759.8/297.2	51	100	3	19:0	alpha
757.7/297.2	51	98	3	19:0	alpha
745.7/297.2	50	98	3	19:0	alpha
743.7/297.2	50	96	3	19:0	alpha
733.7/297.2	49	98	3	19:0	alpha
731.7/297.2	49	96	3	19:0	alpha
719.7/297.2	48	96	3	19:0	alpha

717.7/297.2	48	94	3	19:0	alpha
715.7/297.2	48	92	3	19:0	alpha
705.7/297.2	47	94	3	19:0	alpha
703.7/297.2	47	92	3	19:0	alpha
701.7/297.2	47	90	3	19:0	alpha
689.7/297.2	46	90	3	19:0	alpha
687.7/297.2	46	88	3	19:0	alpha
685.6/297.2	46	86	3	19:0	alpha
677.7/297.2	45	90	3	19:0	alpha
675.7/297.2	45	88	3	19:0	alpha
673.6/297.2	45	86	3	19:0	alpha
663.7/297.2	44	88	3	19:0	alpha
661.6/297.2	44	86	3	19:0	alpha
659.6/297.2	44	84	3	19:0	alpha
657.6/297.2	44	82	3	19:0	alpha
649.6/297.2	43	86	3	19:0	alpha
647.6/297.2	43	84	3	19:0	alpha
645.6/297.2	43	82	3	19:0	alpha
635.6/297.2	42	84	3	19:0	alpha
633.6/297.2	42	82	3	19:0	alpha
631.6/297.2	42	80	3	19:0	alpha
621.6/297.2	41	82	3	19:0	alpha
619.6/297.2	41	80	3	19:0	alpha
617.6/297.2	41	78	3	19:0	alpha
607.6/297.2	40	80	3	19:0	alpha
605.6/297.2	40	78	3	19:0	alpha
603.6/297.2	40	76	3	19:0	alpha
595.6/297.2	39	80	3	19:0	alpha
593.6/297.2	39	78	3	19:0	alpha
591.6/297.2	39	76	3	19:0	alpha
579.6/297.2	38	76	3	19:0	alpha
577.6/297.2	38	74	3	19:0	alpha
575.5/297.2	38	72	3	19:0	alpha
909.9/283.2	62	118	3	18:0	alpha
883.9/283.2	60	116	3	18:0	alpha
881.9/283.2	60	114	3	18:0	alpha
871.9/283.2	59	116	3	18:0	alpha
869.9/283.2	59	114	3	18:0	alpha
867.9/283.2	59	112	3	18:0	alpha
855.9/283.2	58	112	3	18:0	alpha
853.8/283.2	58	110	3	18:0	alpha
843.9/283.2	57	112	3	18:0	alpha
841.8/283.2	57	110	3	18:0	alpha
839.8/283.2	57	108	3	18:0	alpha
827.8/283.2	56	108	3	18:0	alpha
825.8/283.2	56	106	3	18:0	alpha
815.8/283.2	55	108	3	18:0	alpha
813.8/283.2	55	106	3	18:0	alpha
811.8/283.2	55	104	3	18:0	alpha
801.8/283.2	54	106	3	18:0	alpha
799.8/283.2	54	104	3	18:0	alpha
797.8/283.2	54	102	3	18:0	alpha
785.8/283.2	53	102	3	18:0	alpha
783.8/283.2	53	100	3	18:0	alpha
781.7/283.2	53	98	3	18:0	alpha
773.8/283.2	52	102	3	18:0	alpha
771.8/283.2	52	100	3	18:0	alpha
759.8/283.2	51	100	3	18:0	alpha
757.7/283.2	51	98	3	18:0	alpha
745.7/283.2	50	98	3	18:0	alpha
743.7/283.2	50	96	3	18:0	alpha
733.7/283.2	49	98	3	18:0	alpha
731.7/283.2	49	96	3	18:0	alpha
719.7/283.2	48	96	3	18:0	alpha

717.7/283.2	48	94	3	18:0	alpha
715.7/283.2	48	92	3	18:0	alpha
705.7/283.2	47	94	3	18:0	alpha
703.7/283.2	47	92	3	18:0	alpha
701.7/283.2	47	90	3	18:0	alpha
699.7/283.2	46	90	3	18:0	alpha
687.7/283.2	46	88	3	18:0	alpha
685.6/283.2	46	86	3	18:0	alpha
677.7/283.2	45	90	3	18:0	alpha
675.7/283.2	45	88	3	18:0	alpha
673.6/283.2	45	86	3	18:0	alpha
663.7/283.2	44	88	3	18:0	alpha
661.6/283.2	44	86	3	18:0	alpha
659.6/283.2	44	84	3	18:0	alpha
659.6/283.2	44	84	3	18:0	alpha
649.6/283.2	43	86	3	18:0	alpha
647.6/283.2	43	84	3	18:0	alpha
645.6/283.2	43	82	3	18:0	alpha
635.6/283.2	42	84	3	18:0	alpha
633.6/283.2	42	82	3	18:0	alpha
631.6/283.2	42	80	3	18:0	alpha
621.6/283.2	41	82	3	18:0	alpha
619.6/283.2	41	80	3	18:0	alpha
617.8/283.2	41	78	3	18:0	alpha
607.6/283.2	40	80	3	18:0	alpha
605.6/283.2	40	78	3	18:0	alpha
603.6/283.2	40	76	3	18:0	alpha
595.6/283.2	39	80	3	18:0	alpha
593.6/283.2	39	78	3	18:0	alpha
589.6/283.2	39	74	3	18:0	alpha
579.6/283.2	38	76	3	18:0	alpha
577.6/283.2	38	74	3	18:0	alpha
575.5/283.2	38	72	3	18:0	alpha
909.9/281.2	62	118	3	18:1	alpha
883.9/281.2	60	116	3	18:1	alpha
881.9/281.2	60	114	3	18:1	alpha
871.9/281.2	59	116	3	18:1	alpha
869.9/281.2	59	114	3	18:1	alpha
867.9/281.2	59	112	3	18:1	alpha
855.9/281.2	58	112	3	18:1	alpha
853.8/281.2	58	110	3	18:1	alpha
843.9/281.2	57	112	3	18:1	alpha
841.8/281.2	57	110	3	18:1	alpha
839.8/281.2	57	108	3	18:1	alpha
827.8/281.2	56	108	3	18:1	alpha
825.8/281.2	56	106	3	18:1	alpha
815.8/281.2	55	108	3	18:1	alpha
813.8/281.2	55	106	3	18:1	alpha
811.8/281.2	55	104	3	18:1	alpha
801.8/281.2	54	106	3	18:1	alpha
799.8/281.2	54	104	3	18:1	alpha
797.8/281.2	54	102	3	18:1	alpha
787.8/281.2	53	104	3	18:1	alpha
785.8/281.2	53	102	3	18:1	alpha
783.8/281.2	53	100	3	18:1	alpha
773.8/281.2	52	102	3	18:1	alpha
771.8/281.2	52	100	3	18:1	alpha
759.8/281.2	51	100	3	18:1	alpha
757.7/281.2	51	98	3	18:1	alpha
745.7/281.2	50	98	3	18:1	alpha
743.7/281.2	50	96	3	18:1	alpha
733.7/281.2	49	98	3	18:1	alpha
731.7/281.2	49	96	3	18:1	alpha
719.7/281.2	48	96	3	18:1	alpha

717.7/281.2	48	94	3	18:1	alpha
715.7/281.2	48	92	3	18:1	alpha
705.7/281.2	47	94	3	18:1	alpha
703.7/281.2	47	92	3	18:1	alpha
701.7/281.2	47	90	3	18:1	alpha
689.7/281.2	46	90	3	18:1	alpha
687.7/281.2	46	88	3	18:1	alpha
685.6/281.2	46	86	3	18:1	alpha
677.7/281.2	45	90	3	18:1	alpha
675.7/281.2	45	88	3	18:1	alpha
673.6/281.2	45	86	3	18:1	alpha
663.7/281.2	44	88	3	18:1	alpha
661.6/281.2	44	86	3	18:1	alpha
659.6/281.2	44	84	3	18:1	alpha
657.6/281.2	44	82	3	18:1	alpha
649.6/281.2	43	86	3	18:1	alpha
647.6/281.2	43	84	3	18:1	alpha
645.6/281.2	43	82	3	18:1	alpha
635.6/281.2	42	84	3	18:1	alpha
633.6/281.2	42	82	3	18:1	alpha
631.6/281.2	42	80	3	18:1	alpha
621.6/281.2	41	82	3	18:1	alpha
619.6/281.2	41	80	3	18:1	alpha
617.6/281.2	41	78	3	18:1	alpha
607.6/281.2	40	80	3	18:1	alpha
605.6/281.2	40	78	3	18:1	alpha
603.6/281.2	40	76	3	18:1	alpha
595.6/281.2	39	80	3	18:1	alpha
593.6/281.2	39	78	3	18:1	alpha
591.6/281.2	39	76	3	18:1	alpha
579.6/281.2	38	76	3	18:1	alpha
577.6/281.2	38	74	3	18:1	alpha
575.5/281.2	38	72	3	17:0	alpha
909.9/269.2	62	118	3	17:0	alpha
883.9/269.2	60	116	3	17:0	alpha
881.9/269.2	60	114	3	17:0	alpha
871.9/269.2	59	116	3	17:0	alpha
869.9/269.2	59	114	3	17:0	alpha
867.9/269.2	59	112	3	17:0	alpha
855.9/269.2	58	112	3	17:0	alpha
853.8/269.2	58	110	3	17:0	alpha
843.9/269.2	57	112	3	17:0	alpha
841.8/269.2	57	110	3	17:0	alpha
839.8/269.2	57	108	3	17:0	alpha
827.8/269.2	56	108	3	17:0	alpha
825.8/269.2	56	106	3	17:0	alpha
815.8/269.2	55	108	3	17:0	alpha
813.8/269.2	55	106	3	17:0	alpha
811.8/269.2	55	104	3	17:0	alpha
801.8/269.2	54	106	3	17:0	alpha
799.8/269.2	54	104	3	17:0	alpha
797.8/269.2	54	102	3	17:0	alpha
785.8/269.2	53	102	3	17:0	alpha
783.8/269.2	53	100	3	17:0	alpha
781.7/269.2	53	98	3	17:0	alpha
773.8/269.2	52	102	3	17:0	alpha
771.8/269.2	52	100	3	17:0	alpha
759.8/269.2	51	100	3	17:0	alpha
757.7/269.2	51	98	3	17:0	alpha
745.7/269.2	50	98	3	17:0	alpha
743.7/269.2	50	96	3	17:0	alpha
733.7/269.2	49	98	3	17:0	alpha
731.7/269.2	49	96	3	17:0	alpha
719.7/269.2	48	96	3	17:0	alpha

717.7/269.2	48	94	3	17:0	alpha
715.7/269.2	48	92	3	17:0	alpha
705.7/269.2	47	94	3	17:0	alpha
703.7/269.2	47	92	3	17:0	alpha
701.7/269.2	47	90	3	17:0	alpha
689.7/269.2	46	90	3	17:0	alpha
687.7/269.2	46	88	3	17:0	alpha
685.6/269.2	46	86	3	17:0	alpha
677.7/269.2	45	90	3	17:0	alpha
675.7/269.2	45	88	3	17:0	alpha
673.6/269.2	45	86	3	17:0	alpha
663.7/269.2	44	88	3	17:0	alpha
661.6/269.2	44	86	3	17:0	alpha
659.6/269.2	44	84	3	17:0	alpha
659.6/269.2	44	84	3	17:0	alpha
649.6/269.2	43	86	3	17:0	alpha
647.6/269.2	43	84	3	17:0	alpha
645.6/269.2	43	82	3	17:0	alpha
635.6/269.2	42	84	3	17:0	alpha
633.6/269.2	42	82	3	17:0	alpha
631.6/269.2	42	80	3	17:0	alpha
621.6/269.2	41	82	3	17:0	alpha
619.6/269.2	41	80	3	17:0	alpha
617.6/269.2	41	78	3	17:0	alpha
607.6/269.2	40	80	3	17:0	alpha
605.6/269.2	40	78	3	17:0	alpha
603.6/269.2	40	76	3	17:0	alpha
595.6/269.2	39	80	3	17:0	alpha
593.6/269.2	39	78	3	17:0	alpha
589.6/269.2	39	74	3	17:0	alpha
579.6/269.2	38	76	3	17:0	alpha
577.6/269.2	38	74	3	17:0	alpha
575.5/269.2	38	72	3	17:0	alpha
909.9/267.2	62	118	3	17:1	alpha
883.9/267.2	60	116	3	17:1	alpha
881.9/267.2	60	114	3	17:1	alpha
871.9/267.2	59	116	3	17:1	alpha
869.9/267.2	59	114	3	17:1	alpha
867.9/267.2	59	112	3	17:1	alpha
855.9/267.2	58	112	3	17:1	alpha
853.8/267.2	58	110	3	17:1	alpha
843.9/267.2	57	112	3	17:1	alpha
841.8/267.2	57	110	3	17:1	alpha
839.8/267.2	57	108	3	17:1	alpha
827.8/267.2	56	108	3	17:1	alpha
825.8/267.2	56	106	3	17:1	alpha
815.8/267.2	55	108	3	17:1	alpha
813.8/267.2	55	106	3	17:1	alpha
811.8/267.2	55	104	3	17:1	alpha
801.8/267.2	54	106	3	17:1	alpha
799.8/267.2	54	104	3	17:1	alpha
797.8/267.2	54	102	3	17:1	alpha
787.8/267.2	53	104	3	17:1	alpha
785.8/267.2	53	102	3	17:1	alpha
783.8/267.2	53	100	3	17:1	alpha
773.8/267.2	52	102	3	17:1	alpha
771.8/267.2	52	100	3	17:1	alpha
759.8/267.2	51	100	3	17:1	alpha
757.7/267.2	51	98	3	17:1	alpha
745.7/267.2	50	98	3	17:1	alpha
743.7/267.2	50	96	3	17:1	alpha
733.7/267.2	49	98	3	17:1	alpha
731.7/267.2	49	96	3	17:1	alpha
719.7/267.2	48	96	3	17:1	alpha

717.7/267.2	48	94	3	17:1	alpha
715.7/267.2	48	92	3	17:1	alpha
705.7/267.2	47	94	3	17:1	alpha
703.7/267.2	47	92	3	17:1	alpha
701.7/267.2	47	90	3	17:1	alpha
689.7/267.2	46	90	3	17:1	alpha
687.7/267.2	46	88	3	17:1	alpha
685.6/267.2	46	86	3	17:1	alpha
677.7/267.2	45	90	3	17:1	alpha
675.7/267.2	45	88	3	17:1	alpha
673.6/267.2	45	86	3	17:1	alpha
663.7/267.2	44	88	3	17:1	alpha
661.6/267.2	44	86	3	17:1	alpha
659.6/267.2	44	84	3	17:1	alpha
657.6/267.2	44	82	3	17:1	alpha
649.6/267.2	43	86	3	17:1	alpha
647.6/267.2	43	84	3	17:1	alpha
645.6/267.2	43	82	3	17:1	alpha
635.6/267.2	42	84	3	17:1	alpha
633.6/267.2	42	82	3	17:1	alpha
631.6/267.2	42	80	3	17:1	alpha
621.6/267.2	41	82	3	17:1	alpha
619.6/267.2	41	80	3	17:1	alpha
617.6/267.2	41	78	3	17:1	alpha
607.6/267.2	40	80	3	17:1	alpha
605.6/267.2	40	78	3	17:1	alpha
603.6/267.2	40	76	3	17:1	alpha
595.6/267.2	39	80	3	17:1	alpha
593.6/267.2	39	78	3	17:1	alpha
591.6/267.2	39	76	3	17:1	alpha
579.6/267.2	38	76	3	17:1	alpha
577.6/267.2	38	74	3	17:1	alpha
575.5/267.2	38	72	3	17:1	alpha
909.9/255.2	62	118	3	16:0	alpha
883.9/255.2	60	116	3	16:0	alpha
881.9/255.2	60	114	3	16:0	alpha
871.9/255.2	59	116	3	16:0	alpha
869.9/255.2	59	114	3	16:0	alpha
867.9/255.2	59	112	3	16:0	alpha
855.9/255.2	58	112	3	16:0	alpha
853.8/255.2	58	110	3	16:0	alpha
843.9/255.2	57	112	3	16:0	alpha
841.8/255.2	57	110	3	16:0	alpha
839.8/255.2	57	108	3	16:0	alpha
827.8/255.2	56	108	3	16:0	alpha
825.8/255.2	56	106	3	16:0	alpha
815.8/255.2	55	108	3	16:0	alpha
813.8/255.2	55	106	3	16:0	alpha
811.8/255.2	55	104	3	16:0	alpha
801.8/255.2	54	106	3	16:0	alpha
799.8/255.2	54	104	3	16:0	alpha
797.8/255.2	54	102	3	16:0	alpha
785.8/255.2	53	102	3	16:0	alpha
783.8/255.2	53	100	3	16:0	alpha
781.7/255.2	53	98	3	16:0	alpha
773.8/255.2	52	102	3	16:0	alpha
771.8/255.2	52	100	3	16:0	alpha
759.8/255.2	51	100	3	16:0	alpha
757.7/255.2	51	98	3	16:0	alpha
745.7/255.2	50	98	3	16:0	alpha
743.7/255.2	50	96	3	16:0	alpha
733.7/255.2	49	98	3	16:0	alpha
731.7/255.2	49	96	3	16:0	alpha
719.7/255.2	48	96	3	16:0	alpha

717.7/255.2	48	94	3	16:0	alpha
715.7/255.2	48	92	3	16:0	alpha
705.7/255.2	47	94	3	16:0	alpha
703.7/255.2	47	92	3	16:0	alpha
701.7/255.2	47	90	3	16:0	alpha
689.7/255.2	46	90	3	16:0	alpha
687.7/255.2	46	88	3	16:0	alpha
685.8/255.2	46	86	3	16:0	alpha
677.7/255.2	45	90	3	16:0	alpha
675.7/255.2	45	88	3	16:0	alpha
673.8/255.2	45	86	3	16:0	alpha
663.7/255.2	44	88	3	16:0	alpha
661.6/255.2	44	86	3	16:0	alpha
659.8/255.2	44	84	3	16:0	alpha
659.6/255.2	44	84	3	16:0	alpha
649.6/255.2	43	86	3	16:0	alpha
647.6/255.2	43	84	3	16:0	alpha
645.6/255.2	43	82	3	16:0	alpha
635.6/255.2	42	84	3	16:0	alpha
633.6/255.2	42	82	3	16:0	alpha
631.6/255.2	42	80	3	16:0	alpha
621.6/255.2	41	82	3	16:0	alpha
619.6/255.2	41	80	3	16:0	alpha
617.6/255.2	41	78	3	16:0	alpha
607.6/255.2	40	80	3	16:0	alpha
605.6/255.2	40	78	3	16:0	alpha
603.6/255.2	40	76	3	16:0	alpha
595.6/255.2	39	80	3	16:0	alpha
593.6/255.2	39	78	3	16:0	alpha
589.6/255.2	39	74	3	16:0	alpha
579.6/255.2	38	76	3	16:0	alpha
577.6/255.2	38	74	3	16:0	alpha
575.5/255.2	38	72	3	16:0	alpha
909.9/253.2	62	118	3	16:1	alpha
883.9/253.2	60	116	3	16:1	alpha
881.9/253.2	60	114	3	16:1	alpha
871.9/253.2	59	116	3	16:1	alpha
869.9/253.2	59	114	3	16:1	alpha
867.9/253.2	59	112	3	16:1	alpha
855.9/253.2	58	112	3	16:1	alpha
853.8/253.2	58	110	3	16:1	alpha
843.9/253.2	57	112	3	16:1	alpha
841.8/253.2	57	110	3	16:1	alpha
839.8/253.2	57	108	3	16:1	alpha
827.8/253.2	56	108	3	16:1	alpha
825.8/253.2	56	106	3	16:1	alpha
815.8/253.2	55	108	3	16:1	alpha
813.8/253.2	55	106	3	16:1	alpha
811.8/253.2	55	104	3	16:1	alpha
801.8/253.2	54	106	3	16:1	alpha
799.8/253.2	54	104	3	16:1	alpha
797.8/253.2	54	102	3	16:1	alpha
787.8/253.2	53	104	3	16:1	alpha
785.8/253.2	53	102	3	16:1	alpha
783.8/253.2	53	100	3	16:1	alpha
773.8/253.2	52	102	3	16:1	alpha
771.8/253.2	52	100	3	16:1	alpha
759.8/253.2	51	100	3	16:1	alpha
757.7/253.2	51	98	3	16:1	alpha
745.7/253.2	50	98	3	16:1	alpha
743.7/253.2	50	96	3	16:1	alpha
733.7/253.2	49	98	3	16:1	alpha
731.7/253.2	49	96	3	16:1	alpha
719.7/253.2	48	96	3	16:1	alpha

717.7/253.2	48	94	3	16:1	alpha
715.7/253.2	48	92	3	16:1	alpha
705.7/253.2	47	94	3	16:1	alpha
703.7/253.2	47	92	3	16:1	alpha
701.7/253.2	47	90	3	16:1	alpha
689.7/253.2	46	90	3	16:1	alpha
687.7/253.2	46	88	3	16:1	alpha
685.6/253.2	46	86	3	16:1	alpha
677.7/253.2	45	90	3	16:1	alpha
675.7/253.2	45	88	3	16:1	alpha
673.6/253.2	45	86	3	16:1	alpha
663.7/253.2	44	88	3	16:1	alpha
661.6/253.2	44	86	3	16:1	alpha
659.6/253.2	44	84	3	16:1	alpha
657.6/253.2	44	82	3	16:1	alpha
649.6/253.2	43	86	3	16:1	alpha
647.8/253.2	43	84	3	16:1	alpha
645.6/253.2	43	82	3	16:1	alpha
635.6/253.2	42	84	3	16:1	alpha
633.6/253.2	42	82	3	16:1	alpha
631.6/253.2	42	80	3	16:1	alpha
621.8/253.2	41	82	3	16:1	alpha
619.6/253.2	41	80	3	16:1	alpha
617.6/253.2	41	78	3	16:1	alpha
607.6/253.2	40	80	3	16:1	alpha
605.6/253.2	40	78	3	16:1	alpha
603.6/253.2	40	76	3	16:1	alpha
595.6/253.2	39	80	3	16:1	alpha
593.6/253.2	39	78	3	16:1	alpha
591.6/253.2	39	76	3	16:1	alpha
579.6/253.2	38	76	3	16:1	alpha
577.6/253.2	38	74	3	16:1	alpha
575.5/253.2	38	72	3	16:1	alpha
909.9/241.1	62	118	3	15:0	alpha
883.9/241.1	60	116	3	15:0	alpha
881.9/241.1	60	114	3	15:0	alpha
871.9/241.1	59	116	3	15:0	alpha
869.9/241.1	59	114	3	15:0	alpha
867.9/241.1	59	112	3	15:0	alpha
855.9/241.1	58	112	3	15:0	alpha
853.8/241.1	58	110	3	15:0	alpha
843.9/241.1	57	112	3	15:0	alpha
841.8/241.1	57	110	3	15:0	alpha
839.8/241.1	57	108	3	15:0	alpha
827.8/241.1	56	108	3	15:0	alpha
825.8/241.1	56	106	3	15:0	alpha
815.8/241.1	55	108	3	15:0	alpha
813.8/241.1	55	106	3	15:0	alpha
811.8/241.1	55	104	3	15:0	alpha
801.8/241.1	54	106	3	15:0	alpha
799.8/241.1	54	104	3	15:0	alpha
797.8/241.1	54	102	3	15:0	alpha
785.8/241.1	53	102	3	15:0	alpha
783.8/241.1	53	100	3	15:0	alpha
781.7/241.1	53	98	3	15:0	alpha
773.8/241.1	52	102	3	15:0	alpha
771.8/241.1	52	100	3	15:0	alpha
759.8/241.1	51	100	3	15:0	alpha
757.7/241.1	51	98	3	15:0	alpha
745.7/241.1	50	98	3	15:0	alpha
743.7/241.1	50	96	3	15:0	alpha
733.7/241.1	49	98	3	15:0	alpha
731.7/241.1	49	96	3	15:0	alpha
719.7/241.1	48	96	3	15:0	alpha

717.7/241.1	48	94	3	15:0	alpha
715.7/241.1	48	92	3	15:0	alpha
705.7/241.1	47	94	3	15:0	alpha
703.7/241.1	47	92	3	15:0	alpha
701.7/241.1	47	90	3	15:0	alpha
689.7/241.1	46	90	3	15:0	alpha
687.7/241.1	46	88	3	15:0	alpha
685.6/241.1	46	86	3	15:0	alpha
677.7/241.1	45	90	3	15:0	alpha
675.7/241.1	45	88	3	15:0	alpha
673.6/241.1	45	86	3	15:0	alpha
663.7/241.1	44	88	3	15:0	alpha
661.6/241.1	44	86	3	15:0	alpha
659.6/241.1	44	84	3	15:0	alpha
659.6/241.1	44	84	3	15:0	alpha
649.6/241.1	43	86	3	15:0	alpha
647.6/241.1	43	84	3	15:0	alpha
645.6/241.1	43	82	3	15:0	alpha
635.6/241.1	42	84	3	16:0	alpha
633.6/241.1	42	82	3	15:0	alpha
631.6/241.1	42	80	3	15:0	alpha
621.6/241.1	41	82	3	15:0	alpha
619.6/241.1	41	80	3	15:0	alpha
617.6/241.1	41	78	3	15:0	alpha
607.6/241.1	40	80	3	15:0	alpha
605.6/241.1	40	78	3	15:0	alpha
603.6/241.1	40	76	3	15:0	alpha
595.6/241.1	39	80	3	15:0	alpha
593.6/241.1	39	78	3	15:0	alpha
589.6/241.1	38	74	3	15:0	alpha
579.6/241.1	38	76	3	15:0	alpha
577.6/241.1	38	74	3	15:0	alpha
575.5/241.1	38	72	3	15:0	alpha
909.9/239.1	62	118	3	15:1	alpha
883.9/239.1	60	118	3	15:1	alpha
881.9/239.1	60	114	3	15:1	alpha
871.9/239.1	59	116	3	15:1	alpha
869.9/239.1	59	114	3	15:1	alpha
867.9/239.1	59	112	3	15:1	alpha
855.9/239.1	58	112	3	15:1	alpha
853.8/239.1	58	110	3	15:1	alpha
843.9/239.1	57	112	3	15:1	alpha
841.8/239.1	57	110	3	15:1	alpha
839.8/239.1	57	108	3	15:1	alpha
827.8/239.1	56	108	3	15:1	alpha
825.8/239.1	56	106	3	15:1	alpha
815.8/239.1	55	108	3	15:1	alpha
813.8/239.1	55	106	3	15:1	alpha
811.8/239.1	55	104	3	15:1	alpha
801.8/239.1	54	106	3	15:1	alpha
799.8/239.1	54	104	3	15:1	alpha
797.8/239.1	54	102	3	15:1	alpha
787.8/239.1	53	104	3	15:1	alpha
785.8/239.1	53	102	3	15:1	alpha
783.8/239.1	53	100	3	15:1	alpha
773.8/239.1	52	102	3	15:1	alpha
771.8/239.1	52	100	3	15:1	alpha
759.8/239.1	51	100	3	15:1	alpha
757.7/239.1	51	98	3	15:1	alpha
745.7/239.1	50	98	3	15:1	alpha
743.7/239.1	50	96	3	15:1	alpha
733.7/239.1	49	98	3	15:1	alpha
731.7/239.1	49	96	3	15:1	alpha
719.7/239.1	48	96	3	15:1	alpha

717.7/239.1	48	94	3	15:1	alpha
715.7/239.1	48	92	3	15:1	alpha
705.7/239.1	47	94	3	15:1	alpha
703.7/239.1	47	92	3	15:1	alpha
701.7/239.1	47	90	3	15:1	alpha
689.7/239.1	46	90	3	15:1	alpha
687.7/239.1	46	88	3	15:1	alpha
685.6/239.1	46	86	3	15:1	alpha
677.7/239.1	45	90	3	15:1	alpha
675.7/239.1	45	88	3	15:1	alpha
673.6/239.1	45	86	3	15:1	alpha
663.7/239.1	44	88	3	15:1	alpha
661.6/239.1	44	86	3	15:1	alpha
659.6/239.1	44	84	3	15:1	alpha
657.6/239.1	44	82	3	15:1	alpha
649.6/239.1	43	86	3	15:1	alpha
647.6/239.1	43	84	3	15:1	alpha
645.6/239.1	43	82	3	15:1	alpha
635.6/239.1	42	84	3	15:1	alpha
633.6/239.1	42	82	3	15:1	alpha
631.6/239.1	42	80	3	15:1	alpha
621.6/239.1	41	82	3	15:1	alpha
619.6/239.1	41	80	3	15:1	alpha
617.6/239.1	41	78	3	15:1	alpha
607.6/239.1	40	80	3	15:1	alpha
605.6/239.1	40	78	3	15:1	alpha
603.6/239.1	40	76	3	15:1	alpha
595.6/239.1	39	80	3	15:1	alpha
593.6/239.1	39	78	3	15:1	alpha
591.6/239.1	39	76	3	15:1	alpha
579.6/239.1	38	76	3	15:1	alpha
577.6/239.1	38	74	3	15:1	alpha
575.5/239.1	38	72	3	14:0	alpha
909.9/227.1	62	118	3	14:0	alpha
883.9/227.1	60	116	3	14:0	alpha
881.9/227.1	60	114	3	14:0	alpha
871.9/227.1	59	116	3	14:0	alpha
869.9/227.1	59	114	3	14:0	alpha
867.9/227.1	59	112	3	14:0	alpha
855.9/227.1	58	112	3	14:0	alpha
853.8/227.1	58	110	3	14:0	alpha
843.9/227.1	57	112	3	14:0	alpha
841.8/227.1	57	110	3	14:0	alpha
839.8/227.1	57	108	3	14:0	alpha
827.8/227.1	56	108	3	14:0	alpha
825.8/227.1	56	106	3	14:0	alpha
815.8/227.1	55	108	3	14:0	alpha
813.8/227.1	55	106	3	14:0	alpha
811.8/227.1	55	104	3	14:0	alpha
801.8/227.1	54	106	3	14:0	alpha
799.8/227.1	54	104	3	14:0	alpha
797.8/227.1	54	102	3	14:0	alpha
785.8/227.1	53	102	3	14:0	alpha
783.8/227.1	53	100	3	14:0	alpha
781.7/227.1	53	98	3	14:0	alpha
773.8/227.1	52	102	3	14:0	alpha
771.8/227.1	52	100	3	14:0	alpha
759.8/227.1	51	100	3	14:0	alpha
757.7/227.1	51	98	3	14:0	alpha
745.7/227.1	50	98	3	14:0	alpha
743.7/227.1	50	96	3	14:0	alpha
733.7/227.1	49	98	3	14:0	alpha
731.7/227.1	49	96	3	14:0	alpha
719.7/227.1	48	96	3	14:0	alpha

717.7/227.1	48	84	3	14:0	alpha
715.7/227.1	48	92	3	14:0	alpha
705.7/227.1	47	94	3	14:0	alpha
703.7/227.1	47	92	3	14:0	alpha
701.7/227.1	47	90	3	14:0	alpha
689.7/227.1	46	90	3	14:0	alpha
687.7/227.1	46	88	3	14:0	alpha
685.6/227.1	46	86	3	14:0	alpha
677.7/227.1	45	90	3	14:0	alpha
675.7/227.1	45	88	3	14:0	alpha
673.6/227.1	45	86	3	14:0	alpha
663.7/227.1	44	88	3	14:0	alpha
661.6/227.1	44	86	3	14:0	alpha
659.6/227.1	44	84	3	14:0	alpha
659.6/227.1	44	84	3	14:0	alpha
649.6/227.1	43	86	3	14:0	alpha
647.6/227.1	43	84	3	14:0	alpha
645.6/227.1	43	82	3	14:0	alpha
635.6/227.1	42	84	3	14:0	alpha
633.6/227.1	42	82	3	14:0	alpha
631.6/227.1	42	80	3	14:0	alpha
621.6/227.1	41	82	3	14:0	alpha
619.6/227.1	41	80	3	14:0	alpha
617.6/227.1	41	78	3	14:0	alpha
607.6/227.1	40	80	3	14:0	alpha
605.6/227.1	40	78	3	14:0	alpha
603.6/227.1	40	76	3	14:0	alpha
595.6/227.1	39	80	3	14:0	alpha
593.6/227.1	39	78	3	14:0	alpha
589.6/227.1	39	74	3	14:0	alpha
579.6/227.1	38	76	3	14:0	alpha
577.6/227.1	38	74	3	14:0	alpha
575.5/227.1	38	72	3	14:0	alpha
909.9/225.1	62	118	3	14:1	alpha
883.9/225.1	60	116	3	14:1	alpha
881.9/225.1	60	114	3	14:1	alpha
871.9/225.1	59	116	3	14:1	alpha
869.9/225.1	59	114	3	14:1	alpha
867.9/225.1	59	112	3	14:1	alpha
855.9/225.1	58	112	3	14:1	alpha
853.8/225.1	58	110	3	14:1	alpha
843.9/225.1	57	112	3	14:1	alpha
841.8/225.1	57	110	3	14:1	alpha
839.8/225.1	57	108	3	14:1	alpha
827.8/225.1	56	108	3	14:1	alpha
825.8/225.1	56	106	3	14:1	alpha
815.8/225.1	55	108	3	14:1	alpha
813.8/225.1	55	106	3	14:1	alpha
811.8/225.1	55	104	3	14:1	alpha
801.8/225.1	54	106	3	14:1	alpha
799.8/225.1	54	104	3	14:1	alpha
797.8/225.1	54	102	3	14:1	alpha
787.8/225.1	53	104	3	14:1	alpha
785.8/225.1	53	102	3	14:1	alpha
783.8/225.1	53	100	3	14:1	alpha
773.8/225.1	52	102	3	14:1	alpha
771.8/225.1	52	100	3	14:1	alpha
759.8/225.1	51	100	3	14:1	alpha
757.7/225.1	51	98	3	14:1	alpha
745.7/225.1	50	98	3	14:1	alpha
743.7/225.1	50	96	3	14:1	alpha
733.7/225.1	49	98	3	14:1	alpha
731.7/225.1	49	96	3	14:1	alpha
719.7/225.1	48	96	3	14:1	alpha

717.7/225.1	46	94	3	14:1	alpha
715.7/225.1	48	92	3	14:1	alpha
705.7/225.1	47	94	3	14:1	alpha
703.7/225.1	47	92	3	14:1	alpha
701.7/225.1	47	90	3	14:1	alpha
689.7/225.1	46	90	3	14:1	alpha
687.7/225.1	46	88	3	14:1	alpha
685.6/225.1	46	86	3	14:1	alpha
677.7/225.1	45	90	3	14:1	alpha
675.7/225.1	45	88	3	14:1	alpha
673.6/225.1	45	86	3	14:1	alpha
663.7/225.1	44	88	3	14:1	alpha
661.6/225.1	44	86	3	14:1	alpha
659.6/225.1	44	84	3	14:1	alpha
657.6/225.1	44	82	3	14:1	alpha
649.6/225.1	43	86	3	14:1	alpha
647.6/225.1	43	84	3	14:1	alpha
645.6/225.1	43	82	3	14:1	alpha
635.6/225.1	42	84	3	14:1	alpha
633.6/225.1	42	82	3	14:1	alpha
631.6/225.1	42	80	3	14:1	alpha
621.6/225.1	41	82	3	14:1	alpha
619.6/225.1	41	80	3	14:1	alpha
617.6/225.1	41	78	3	14:1	alpha
607.6/225.1	40	80	3	14:1	alpha
605.6/225.1	40	78	3	14:1	alpha
603.6/225.1	40	76	3	14:1	alpha
595.6/225.1	39	80	3	14:1	alpha
593.6/225.1	39	78	3	14:1	alpha
591.6/225.1	39	76	3	14:1	alpha
579.6/225.1	38	76	3	14:1	alpha
577.6/225.1	38	74	3	14:1	alpha
575.5/225.1	38	72	3	14:1	alpha
909.9/213.1	62	118	3	13:0	alpha
883.9/213.1	60	116	3	13:0	alpha
881.9/213.1	60	114	3	13:0	alpha
871.9/213.1	59	116	3	13:0	alpha
869.9/213.1	59	114	3	13:0	alpha
867.9/213.1	59	112	3	13:0	alpha
856.9/213.1	58	112	3	13:0	alpha
853.8/213.1	58	110	3	13:0	alpha
843.9/213.1	57	112	3	13:0	alpha
841.8/213.1	57	110	3	13:0	alpha
839.8/213.1	57	108	3	13:0	alpha
827.8/213.1	56	108	3	13:0	alpha
825.8/213.1	56	106	3	13:0	alpha
815.8/213.1	55	108	3	13:0	alpha
813.8/213.1	55	106	3	13:0	alpha
811.8/213.1	55	104	3	13:0	alpha
801.8/213.1	54	106	3	13:0	alpha
799.8/213.1	54	104	3	13:0	alpha
797.8/213.1	54	102	3	13:0	alpha
785.8/213.1	53	102	3	13:0	alpha
783.8/213.1	53	100	3	13:0	alpha
781.7/213.1	53	98	3	13:0	alpha
773.8/213.1	52	102	3	13:0	alpha
771.8/213.1	52	100	3	13:0	alpha
769.8/213.1	51	100	3	13:0	alpha
757.7/213.1	51	98	3	13:0	alpha
745.7/213.1	50	98	3	13:0	alpha
743.7/213.1	50	96	3	13:0	alpha
733.7/213.1	49	98	3	13:0	alpha
731.7/213.1	49	96	3	13:0	alpha
719.7/213.1	48	96	3	13:0	alpha

717.7/213.1	48	94	3	13:0	alpha
715.7/213.1	48	92	3	13:0	alpha
705.7/213.1	47	94	3	13:0	alpha
703.7/213.1	47	92	3	13:0	alpha
701.7/213.1	47	90	3	13:0	alpha
689.7/213.1	46	90	3	13:0	alpha
687.7/213.1	46	88	3	13:0	alpha
685.6/213.1	46	86	3	13:0	alpha
677.7/213.1	45	90	3	13:0	alpha
675.7/213.1	45	88	3	13:0	alpha
673.6/213.1	45	86	3	13:0	alpha
663.7/213.1	44	88	3	13:0	alpha
661.6/213.1	44	86	3	13:0	alpha
659.6/213.1	44	84	3	13:0	alpha
659.6/213.1	44	84	3	13:0	alpha
648.6/213.1	43	88	3	13:0	alpha
647.6/213.1	43	84	3	13:0	alpha
645.6/213.1	43	82	3	13:0	alpha
635.6/213.1	42	84	3	13:0	alpha
633.6/213.1	42	82	3	13:0	alpha
631.6/213.1	42	80	3	13:0	alpha
621.6/213.1	41	82	3	13:0	alpha
619.6/213.1	41	80	3	13:0	alpha
617.6/213.1	41	78	3	13:0	alpha
607.6/213.1	40	80	3	13:0	alpha
605.6/213.1	40	78	3	13:0	alpha
603.6/213.1	40	76	3	13:0	alpha
595.6/213.1	39	80	3	13:0	alpha
593.6/213.1	39	78	3	13:0	alpha
589.6/213.1	39	74	3	13:0	alpha
579.6/213.1	38	76	3	13:0	alpha
577.8/213.1	38	74	3	13:0	alpha
575.5/213.1	38	72	3	13:0	alpha
909.9/199.1	62	118	3	12:0	alpha
883.9/199.1	60	116	3	12:0	alpha
881.9/199.1	60	114	3	12:0	alpha
871.9/199.1	59	118	3	12:0	alpha
869.9/199.1	59	114	3	12:0	alpha
867.9/199.1	59	112	3	12:0	alpha
855.9/199.1	58	112	3	12:0	alpha
853.8/199.1	58	110	3	12:0	alpha
843.9/199.1	57	112	3	12:0	alpha
841.8/199.1	57	110	3	12:0	alpha
839.8/199.1	57	108	3	12:0	alpha
827.8/199.1	56	108	3	12:0	alpha
825.8/199.1	56	106	3	12:0	alpha
815.8/199.1	55	108	3	12:0	alpha
813.8/199.1	55	106	3	12:0	alpha
811.8/199.1	55	104	3	12:0	alpha
801.8/199.1	54	106	3	12:0	alpha
799.8/199.1	54	104	3	12:0	alpha
797.8/199.1	54	102	3	12:0	alpha
787.8/199.1	53	104	3	12:0	alpha
785.8/199.1	53	102	3	12:0	alpha
783.8/199.1	53	100	3	12:0	alpha
773.8/199.1	52	102	3	12:0	alpha
771.8/199.1	52	100	3	12:0	alpha
759.8/199.1	51	100	3	12:0	alpha
757.7/199.1	51	98	3	12:0	alpha
745.7/199.1	50	98	3	12:0	alpha
743.7/199.1	50	96	3	12:0	alpha
733.7/199.1	49	98	3	12:0	alpha
731.7/199.1	49	96	3	12:0	alpha
719.7/199.1	48	96	3	12:0	alpha

717.7/199.1	48	94	3	12:0	alpha
715.7/199.1	48	92	3	12:0	alpha
705.7/199.1	47	94	3	12:0	alpha
703.7/199.1	47	92	3	12:0	alpha
701.7/199.1	47	90	3	12:0	alpha
689.7/199.1	46	90	3	12:0	alpha
687.7/199.1	46	88	3	12:0	alpha
685.6/199.1	46	86	3	12:0	alpha
677.7/199.1	45	90	3	12:0	alpha
675.7/199.1	45	88	3	12:0	alpha
673.6/199.1	45	86	3	12:0	alpha
663.7/199.1	44	88	3	12:0	alpha
661.6/199.1	44	86	3	12:0	alpha
659.6/199.1	44	84	3	12:0	alpha
657.6/199.1	44	82	3	12:0	alpha
649.6/199.1	43	86	3	12:0	alpha
647.6/199.1	43	84	3	12:0	alpha
645.6/199.1	43	82	3	12:0	alpha
635.6/199.1	42	84	3	12:0	alpha
633.6/199.1	42	82	3	12:0	alpha
631.6/199.1	42	80	3	12:0	alpha
621.6/199.1	41	82	3	12:0	alpha
619.6/199.1	41	80	3	12:0	alpha
617.6/199.1	41	78	3	12:0	alpha
607.6/199.1	40	80	3	12:0	alpha
605.6/199.1	40	78	3	12:0	alpha
603.6/199.1	40	76	3	12:0	alpha
595.6/199.1	39	80	3	12:0	alpha
593.6/199.1	39	78	3	12:0	alpha
591.6/199.1	39	76	3	12:0	alpha
579.6/199.1	38	76	3	12:0	alpha
577.6/199.1	38	74	3	12:0	alpha
575.5/199.1	38	72	3	12:0	alpha
909.9/197.1	62	118	3	12:1	alpha
883.9/197.1	60	116	3	12:1	alpha
881.9/197.1	60	114	3	12:1	alpha
871.9/197.1	59	116	3	12:1	alpha
869.9/197.1	59	114	3	12:1	alpha
867.9/197.1	59	112	3	12:1	alpha
855.9/197.1	58	112	3	12:1	alpha
853.8/197.1	58	110	3	12:1	alpha
843.9/197.1	57	112	3	12:1	alpha
841.8/197.1	57	110	3	12:1	alpha
839.8/197.1	57	108	3	12:1	alpha
827.8/197.1	56	108	3	12:1	alpha
825.8/197.1	56	106	3	12:1	alpha
815.8/197.1	55	108	3	12:1	alpha
813.8/197.1	55	106	3	12:1	alpha
811.8/197.1	55	104	3	12:1	alpha
801.8/197.1	54	106	3	12:1	alpha
799.8/197.1	54	104	3	12:1	alpha
797.8/197.1	54	102	3	12:1	alpha
785.8/197.1	53	102	3	12:1	alpha
783.8/197.1	53	100	3	12:1	alpha
781.7/197.1	53	98	3	12:1	alpha
773.8/197.1	52	102	3	12:1	alpha
771.8/197.1	52	100	3	12:1	alpha
759.8/197.1	51	100	3	12:1	alpha
757.7/197.1	51	98	3	12:1	alpha
745.7/197.1	50	98	3	12:1	alpha
743.7/197.1	50	96	3	12:1	alpha
733.7/197.1	49	98	3	12:1	alpha
731.7/197.1	49	96	3	12:1	alpha
719.7/197.1	48	96	3	12:1	alpha

717.7/197.1	48	84	3	12:1	alpha
715.7/197.1	48	92	3	12:1	alpha
705.7/197.1	47	94	3	12:1	alpha
703.7/197.1	47	92	3	12:1	alpha
701.7/197.1	47	90	3	12:1	alpha
689.7/197.1	46	90	3	12:1	alpha
687.7/197.1	46	88	3	12:1	alpha
685.6/197.1	46	86	3	12:1	alpha
677.7/197.1	45	90	3	12:1	alpha
675.7/197.1	45	88	3	12:1	alpha
673.6/197.1	45	88	3	12:1	alpha
663.7/197.1	44	88	3	12:1	alpha
661.6/197.1	44	86	3	12:1	alpha
659.6/197.1	44	84	3	12:1	alpha
659.6/197.1	44	84	3	12:1	alpha
649.6/197.1	43	86	3	12:1	alpha
647.6/197.1	43	84	3	12:1	alpha
645.6/197.1	43	82	3	12:1	alpha
635.6/197.1	42	84	3	12:1	alpha
633.6/197.1	42	82	3	12:1	alpha
631.6/197.1	42	80	3	12:1	alpha
621.6/197.1	41	82	3	12:1	alpha
619.6/197.1	41	80	3	12:1	alpha
617.6/197.1	41	78	3	12:1	alpha
607.6/197.1	40	80	3	12:1	alpha
605.6/197.1	40	78	3	12:1	alpha
603.6/197.1	40	76	3	12:1	alpha
595.6/197.1	39	80	3	12:1	alpha
593.6/197.1	39	78	3	12:1	alpha
589.6/197.1	39	74	3	12:1	alpha
579.6/197.1	38	76	3	12:1	alpha
577.6/197.1	38	74	3	12:1	alpha
575.5/197.1	38	72	3	12:1	alpha
909.9/185.1	62	118	3	11:0	alpha
883.9/185.1	60	116	3	11:0	alpha
881.9/185.1	60	114	3	11:0	alpha
871.9/185.1	59	116	3	11:0	alpha
869.9/185.1	59	114	3	11:0	alpha
867.9/185.1	59	112	3	11:0	alpha
855.9/185.1	58	112	3	11:0	alpha
853.8/185.1	58	110	3	11:0	alpha
843.9/185.1	57	112	3	11:0	alpha
841.8/185.1	57	110	3	11:0	alpha
839.8/185.1	57	108	3	11:0	alpha
827.8/185.1	56	108	3	11:0	alpha
825.8/185.1	56	106	3	11:0	alpha
815.8/185.1	55	108	3	11:0	alpha
813.8/185.1	55	106	3	11:0	alpha
811.8/185.1	55	104	3	11:0	alpha
801.8/185.1	54	106	3	11:0	alpha
799.8/185.1	54	104	3	11:0	alpha
797.8/185.1	54	102	3	11:0	alpha
787.8/185.1	53	104	3	11:0	alpha
785.8/185.1	53	102	3	11:0	alpha
783.8/185.1	53	100	3	11:0	alpha
773.8/185.1	52	102	3	11:0	alpha
771.8/185.1	52	100	3	11:0	alpha
759.8/185.1	51	100	3	11:0	alpha
757.7/185.1	51	98	3	11:0	alpha
745.7/185.1	50	98	3	11:0	alpha
743.7/185.1	50	96	3	11:0	alpha
733.7/185.1	49	98	3	11:0	alpha
731.7/185.1	49	96	3	11:0	alpha
719.7/185.1	48	96	3	11:0	alpha

717.7/185.1	48	94	3	11:0	alpha
715.7/185.1	48	92	3	11:0	alpha
705.7/185.1	47	94	3	11:0	alpha
703.7/185.1	47	92	3	11:0	alpha
701.7/185.1	47	90	3	11:0	alpha
689.7/185.1	46	90	3	11:0	alpha
687.7/185.1	46	88	3	11:0	alpha
685.6/185.1	46	86	3	11:0	alpha
677.7/185.1	45	90	3	11:0	alpha
675.7/185.1	45	88	3	11:0	alpha
673.6/185.1	45	86	3	11:0	alpha
663.7/185.1	44	88	3	11:0	alpha
661.6/185.1	44	86	3	11:0	alpha
659.6/185.1	44	84	3	11:0	alpha
657.6/185.1	44	82	3	11:0	alpha
649.6/185.1	43	86	3	11:0	alpha
647.6/185.1	43	84	3	11:0	alpha
645.6/185.1	43	82	3	11:0	alpha
635.6/185.1	42	84	3	11:0	alpha
633.6/185.1	42	82	3	11:0	alpha
631.6/185.1	42	80	3	11:0	alpha
621.6/185.1	41	82	3	11:0	alpha
619.6/185.1	41	80	3	11:0	alpha
617.6/185.1	41	78	3	11:0	alpha
607.6/185.1	40	80	3	11:0	alpha
605.6/185.1	40	78	3	11:0	alpha
603.6/185.1	40	76	3	11:0	alpha
595.6/185.1	39	80	3	11:0	alpha
593.6/185.1	39	78	3	11:0	alpha
591.6/185.1	39	76	3	11:0	alpha
579.6/185.1	38	76	3	11:0	alpha
577.6/185.1	38	74	3	11:0	alpha
575.5/185.1	38	72	3	11:0	alpha
909.9/171.1	62	118	3	10:0	alpha
883.9/171.1	60	116	3	10:0	alpha
881.9/171.1	60	114	3	10:0	alpha
871.9/171.1	59	116	3	10:0	alpha
869.9/171.1	59	114	3	10:0	alpha
867.9/171.1	59	112	3	10:0	alpha
855.9/171.1	58	112	3	10:0	alpha
853.8/171.1	58	110	3	10:0	alpha
843.9/171.1	57	112	3	10:0	alpha
841.8/171.1	57	110	3	10:0	alpha
839.8/171.1	57	108	3	10:0	alpha
827.8/171.1	56	108	3	10:0	alpha
825.8/171.1	56	106	3	10:0	alpha
815.8/171.1	55	108	3	10:0	alpha
813.8/171.1	55	106	3	10:0	alpha
811.8/171.1	55	104	3	10:0	alpha
801.8/171.1	54	106	3	10:0	alpha
799.8/171.1	54	104	3	10:0	alpha
797.8/171.1	54	102	3	10:0	alpha
785.8/171.1	53	102	3	10:0	alpha
783.8/171.1	53	100	3	10:0	alpha
781.7/171.1	53	98	3	10:0	alpha
773.8/171.1	52	102	3	10:0	alpha
771.8/171.1	52	100	3	10:0	alpha
759.8/171.1	51	100	3	10:0	alpha
757.7/171.1	51	98	3	10:0	alpha
745.7/171.1	50	98	3	10:0	alpha
743.7/171.1	50	96	3	10:0	alpha
733.7/171.1	49	98	3	10:0	alpha
731.7/171.1	49	96	3	10:0	alpha
719.7/171.1	48	96	3	10:0	alpha

717.7/171.1	48	94	3	10:0	alpha
715.7/171.1	48	92	3	10:0	alpha
705.7/171.1	47	94	3	10:0	alpha
703.7/171.1	47	92	3	10:0	alpha
701.7/171.1	47	90	3	10:0	alpha
689.7/171.1	46	90	3	10:0	alpha
687.7/171.1	46	88	3	10:0	alpha
685.6/171.1	46	86	3	10:0	alpha
677.7/171.1	45	90	3	10:0	alpha
675.7/171.1	45	88	3	10:0	alpha
673.6/171.1	45	86	3	10:0	alpha
663.7/171.1	44	88	3	10:0	alpha
661.6/171.1	44	86	3	10:0	alpha
659.6/171.1	44	84	3	10:0	alpha
659.6/171.1	44	84	3	10:0	alpha
649.6/171.1	43	86	3	10:0	alpha
647.6/171.1	43	84	3	10:0	alpha
645.6/171.1	43	82	3	10:0	alpha
635.6/171.1	42	84	3	10:0	alpha
633.6/171.1	42	82	3	10:0	alpha
631.6/171.1	42	80	3	10:0	alpha
621.6/171.1	41	82	3	10:0	alpha
619.6/171.1	41	80	3	10:0	alpha
617.6/171.1	41	78	3	10:0	alpha
607.6/171.1	40	80	3	10:0	alpha
605.6/171.1	40	78	3	10:0	alpha
603.6/171.1	40	76	3	10:0	alpha
595.6/171.1	39	80	3	10:0	alpha
593.6/171.1	39	78	3	10:0	alpha
589.6/171.1	39	74	3	10:0	alpha
579.6/171.1	38	76	3	10:0	alpha
577.6/171.1	38	74	3	10:0	alpha
575.5/171.1	38	72	3	10:0	alpha
409.4/283.2	26	50	3	18:0	alpha
409.4/283.2	26	50	3	18:0	alpha
437.4/283.2	28	54	3	18:0	alpha
439.4/283.2	28	56	3	18:0	alpha
463.4/283.2	30	56	3	18:0	alpha
465.4/283.2	30	58	3	18:0	alpha
467.4/283.2	30	60	3	18:0	alpha
481.5/283.2	31	62	3	18:0	alpha
491.4/283.2	32	60	3	18:0	alpha
493.5/283.2	32	62	3	18:0	alpha
495.5/283.2	32	64	3	18:0	alpha
509.5/283.2	33	66	3	18:0	alpha
519.5/283.2	34	64	3	18:0	alpha
521.5/283.2	34	66	3	18:0	alpha
523.5/283.2	34	68	3	18:0	alpha
545.5/283.2	36	66	3	18:0	alpha
547.5/283.2	36	68	3	18:0	alpha
549.5/283.2	36	70	3	18:0	alpha
551.5/283.2	36	72	3	18:0	alpha
409.4/281.2	26	50	3	18:1	alpha
409.4/281.2	26	50	3	18:1	alpha
437.4/281.2	28	54	3	18:1	alpha
439.4/281.2	28	56	3	18:1	alpha
463.4/281.2	30	56	3	18:1	alpha
465.4/281.2	30	58	3	18:1	alpha
467.4/281.2	30	60	3	18:1	alpha
481.5/281.2	31	62	3	18:1	alpha
491.4/281.2	32	60	3	18:1	alpha
493.5/281.2	32	62	3	18:1	alpha
495.5/281.2	32	64	3	18:1	alpha
509.5/281.2	33	66	3	18:1	alpha

519.5/281.2	34	64	3	18:1	alpha
521.5/281.2	34	66	3	18:1	alpha
523.5/281.2	34	68	3	18:1	alpha
545.5/281.2	36	66	3	18:1	alpha
547.5/281.2	36	68	3	18:1	alpha
549.5/281.2	36	70	3	18:1	alpha
551.5/281.2	36	72	3	18:1	alpha
409.4/269.2	26	50	3	17:0	alpha
409.4/269.2	26	50	3	17:0	alpha
437.4/269.2	28	54	3	17:0	alpha
439.4/269.2	28	56	3	17:0	alpha
463.4/269.2	30	56	3	17:0	alpha
465.4/269.2	30	58	3	17:0	alpha
467.4/269.2	30	60	3	17:0	alpha
481.5/269.2	31	62	3	17:0	alpha
491.4/269.2	32	60	3	17:0	alpha
493.5/269.2	32	62	3	17:0	alpha
495.5/269.2	32	64	3	17:0	alpha
509.5/269.2	33	66	3	17:0	alpha
519.5/269.2	34	64	3	17:0	alpha
521.5/269.2	34	66	3	17:0	alpha
523.5/269.2	34	68	3	17:0	alpha
545.5/269.2	36	66	3	17:0	alpha
547.5/269.2	36	68	3	17:0	alpha
549.5/269.2	36	70	3	17:0	alpha
551.5/269.2	36	72	3	17:0	alpha
409.4/255.2	26	50	3	16:0	alpha
409.4/255.2	26	50	3	16:0	alpha
437.4/255.2	28	54	3	16:0	alpha
439.4/255.2	28	56	3	16:0	alpha
463.4/255.2	30	56	3	16:0	alpha
465.4/255.2	30	58	3	16:0	alpha
467.4/255.2	30	60	3	16:0	alpha
481.5/255.2	31	62	3	16:0	alpha
491.4/255.2	32	60	3	16:0	alpha
493.5/255.2	32	62	3	16:0	alpha
495.5/255.2	32	64	3	16:0	alpha
509.5/255.2	33	66	3	16:0	alpha
519.5/255.2	34	64	3	16:0	alpha
521.5/255.2	34	66	3	16:0	alpha
523.5/255.2	34	68	3	16:0	alpha
545.5/255.2	36	66	3	16:0	alpha
547.5/255.2	36	68	3	16:0	alpha
549.5/255.2	36	70	3	16:0	alpha
551.5/255.2	36	72	3	16:0	alpha
409.4/253.2	26	50	3	16:1	alpha
409.4/253.2	26	50	3	16:1	alpha
437.4/253.2	28	54	3	16:1	alpha
439.4/253.2	28	56	3	16:1	alpha
463.4/253.2	30	56	3	16:1	alpha
465.4/253.2	30	58	3	16:1	alpha
467.4/253.2	30	60	3	16:1	alpha
481.5/253.2	31	62	3	16:1	alpha
491.4/253.2	32	60	3	16:1	alpha
493.5/253.2	32	62	3	16:1	alpha
495.5/253.2	32	64	3	16:1	alpha
509.5/253.2	33	66	3	16:1	alpha
519.5/253.2	34	64	3	16:1	alpha
521.5/253.2	34	66	3	16:1	alpha
523.5/253.2	34	68	3	16:1	alpha
545.5/253.2	36	66	3	16:1	alpha
547.5/253.2	36	68	3	16:1	alpha
549.5/253.2	36	70	3	16:1	alpha
551.5/253.2	36	72	3	16:1	alpha

409.4/241.1	26	50	3	15:0	alpha
409.4/241.1	26	50	3	15:0	alpha
437.4/241.1	28	54	3	15:0	alpha
439.4/241.1	28	56	3	15:0	alpha
463.4/241.1	30	58	3	15:0	alpha
465.4/241.1	30	58	3	15:0	alpha
467.4/241.1	30	60	3	15:0	alpha
481.5/241.1	31	62	3	15:0	alpha
491.4/241.1	32	60	3	15:0	alpha
493.5/241.1	32	62	3	15:0	alpha
495.5/241.1	32	64	3	15:0	alpha
509.5/241.1	33	66	3	15:0	alpha
519.5/241.1	34	64	3	15:0	alpha
521.5/241.1	34	66	3	15:0	alpha
523.5/241.1	34	68	3	15:0	alpha
545.5/241.1	36	66	3	15:0	alpha
547.5/241.1	36	68	3	15:0	alpha
549.5/241.1	36	70	3	15:0	alpha
551.5/241.1	36	72	3	15:0	alpha
409.4/227.1	26	50	3	14:0	alpha
409.4/227.1	26	50	3	14:0	alpha
437.4/227.1	28	54	3	14:0	alpha
439.4/227.1	28	56	3	14:0	alpha
463.4/227.1	30	56	3	14:0	alpha
465.4/227.1	30	58	3	14:0	alpha
467.4/227.1	30	60	3	14:0	alpha
481.5/227.1	31	62	3	14:0	alpha
491.4/227.1	32	60	3	14:0	alpha
493.5/227.1	32	62	3	14:0	alpha
495.5/227.1	32	64	3	14:0	alpha
509.5/227.1	33	66	3	14:0	alpha
519.5/227.1	34	64	3	14:0	alpha
521.5/227.1	34	66	3	14:0	alpha
523.5/227.1	34	68	3	14:0	alpha
545.5/227.1	36	66	3	14:0	alpha
547.5/227.1	36	68	3	14:0	alpha
549.5/227.1	36	70	3	14:0	alpha
551.5/227.1	36	72	3	14:0	alpha
409.4/225.1	26	50	3	14:1	alpha
409.4/225.1	26	50	3	14:1	alpha
437.4/225.1	28	54	3	14:1	alpha
439.4/225.1	28	56	3	14:1	alpha
463.4/225.1	30	56	3	14:1	alpha
465.4/225.1	30	58	3	14:1	alpha
467.4/225.1	30	60	3	14:1	alpha
481.5/225.1	31	62	3	14:1	alpha
491.4/225.1	32	60	3	14:1	alpha
493.5/225.1	32	62	3	14:1	alpha
495.5/225.1	32	64	3	14:1	alpha
509.5/225.1	33	66	3	14:1	alpha
519.5/225.1	34	64	3	14:1	alpha
521.5/225.1	34	66	3	14:1	alpha
523.5/225.1	34	68	3	14:1	alpha
545.5/225.1	36	66	3	14:1	alpha
547.5/225.1	36	68	3	14:1	alpha
549.5/225.1	36	70	3	14:1	alpha
551.5/225.1	36	72	3	14:1	alpha
409.4/199.1	26	50	3	12:0	alpha
409.4/199.1	26	50	3	12:0	alpha
437.4/199.1	28	54	3	12:0	alpha
439.4/199.1	28	56	3	12:0	alpha
463.4/199.1	30	56	3	12:0	alpha
465.4/199.1	30	58	3	12:0	alpha
467.4/199.1	30	60	3	12:0	alpha

481.5/199.1	31	62	3	12:0	alpha
491.4/199.1	32	60	3	12:0	alpha
493.5/199.1	32	62	3	12:0	alpha
495.5/199.1	32	64	3	12:0	alpha
509.5/199.1	33	66	3	12:0	alpha
519.5/199.1	34	64	3	12:0	alpha
521.5/199.1	34	66	3	12:0	alpha
523.5/199.1	34	68	3	12:0	alpha
545.5/199.1	36	66	3	12:0	alpha
547.5/199.1	36	68	3	12:0	alpha
549.5/199.1	36	70	3	12:0	alpha
551.5/199.1	36	72	3	12:0	alpha
409.4/197.1	26	50	3	12:1	alpha
409.4/197.1	26	50	3	12:1	alpha
437.4/197.1	28	54	3	12:1	alpha
439.4/197.1	28	56	3	12:1	alpha
463.4/197.1	30	56	3	12:1	alpha
465.4/197.1	30	58	3	12:1	alpha
467.4/197.1	30	60	3	12:1	alpha
481.5/197.1	31	62	3	12:1	alpha
491.4/197.1	32	60	3	12:1	alpha
493.5/197.1	32	62	3	12:1	alpha
495.5/197.1	32	64	3	12:1	alpha
509.5/197.1	33	66	3	12:1	alpha
519.5/197.1	34	64	3	12:1	alpha
521.5/197.1	34	66	3	12:1	alpha
523.5/197.1	34	68	3	12:1	alpha
545.5/197.1	36	66	3	12:1	alpha
547.5/197.1	36	68	3	12:1	alpha
549.5/197.1	36	70	3	12:1	alpha
551.5/197.1	36	72	3	12:1	alpha
409.4/171.1	26	50	3	10:0	alpha
409.4/171.1	26	50	3	10:0	alpha
437.4/171.1	28	54	3	10:0	alpha
439.4/171.1	28	56	3	10:0	alpha
463.4/171.1	30	56	3	10:0	alpha
465.4/171.1	30	58	3	10:0	alpha
467.4/171.1	30	60	3	10:0	alpha
481.5/171.1	31	62	3	10:0	alpha
491.4/171.1	32	60	3	10:0	alpha
493.5/171.1	32	62	3	10:0	alpha
495.5/171.1	32	64	3	10:0	alpha
509.5/171.1	33	66	3	10:0	alpha
519.5/171.1	34	64	3	10:0	alpha
521.5/171.1	34	66	3	10:0	alpha
523.5/171.1	34	68	3	10:0	alpha
545.5/171.1	36	66	3	10:0	alpha
547.5/171.1	36	68	3	10:0	alpha
549.5/171.1	36	70	3	10:0	alpha
551.5/171.1	36	72	3	10:0	alpha
409.4/169.1	26	50	3	10:1	alpha
409.4/169.1	26	50	3	10:1	alpha
437.4/169.1	28	54	3	10:1	alpha
439.4/169.1	28	56	3	10:1	alpha
463.4/169.1	30	56	3	10:1	alpha
465.4/169.1	30	58	3	10:1	alpha
467.4/169.1	30	60	3	10:1	alpha
481.5/169.1	31	62	3	10:1	alpha
491.4/169.1	32	60	3	10:1	alpha
493.5/169.1	32	62	3	10:1	alpha
495.5/169.1	32	64	3	10:1	alpha
509.5/169.1	33	66	3	10:1	alpha
519.5/169.1	34	64	3	10:1	alpha
521.5/169.1	34	66	3	10:1	alpha

523.5/169.1	34	68	3	10:1	alpha
545.5/169.1	36	66	3	10:1	alpha
547.5/169.1	36	68	3	10:1	alpha
549.5/169.1	36	70	3	10:1	alpha
551.5/169.1	36	72	3	10:1	alpha
409.4/143.1	28	50	3	8:0	alpha
409.4/143.1	26	50	3	8:0	alpha
437.4/143.1	28	54	3	8:0	alpha
439.4/143.1	28	56	3	8:0	alpha
463.4/143.1	30	56	3	8:0	alpha
465.4/143.1	30	58	3	8:0	alpha
467.4/143.1	30	60	3	8:0	alpha
481.5/143.1	31	62	3	8:0	alpha
491.4/143.1	32	60	3	8:0	alpha
493.5/143.1	32	62	3	8:0	alpha
495.5/143.1	32	64	3	8:0	alpha
509.5/143.1	33	66	3	8:0	alpha
519.5/143.1	34	64	3	8:0	alpha
521.5/143.1	34	66	3	8:0	alpha
523.5/143.1	34	68	3	8:0	alpha
545.5/143.1	36	66	3	8:0	alpha
547.5/143.1	36	68	3	8:0	alpha
549.5/143.1	36	70	3	8:0	alpha
551.5/143.1	36	72	3	8:0	alpha

ESI/MS is performed on the test sample, and a profile is generated for a set of mycolic acids in the test sample. The set of mycolic acids can include certain mycolic acids selected from those set forth in Supplementary Table 1. If desired, the entire group of mycolic acids of Supplementary Table 1 can be investigated;

5 alternatively, a subset can be investigated. In a preferred embodiment, a set of C26- α -mycolic acids is investigated. In another preferred embodiment, a set of C24- α -mycolic acids is investigated. In yet another preferred embodiment, a set of C22- α -mycolic acids is investigated.

In certain embodiments of the invention, the profile of the set of mycolic acids in the test sample is examined for a profile consistent with the presence of
10 C26- α -mycolic acids. The presence of C26- α -mycolic acids as shown by the profile is indicative of the presence of *Mycobacterium tuberculosis*, and is therefore diagnostic for an active tuberculosis infection in the individual from whom the test sample was obtained.

15 In other embodiments of the invention, the profile of the set of mycolic acids in the test sample is compared to a profile of the set of the same mycolic acids in a positive sample. A "positive sample," as used herein, refers to a sample that is known to contain *Mycobacterium tuberculosis*. Representative positive samples include comparable positive samples (e.g., samples that are derived from the same
20 source (sputum, lung tissue) as the test sample) as well as positive samples that have been artificially generated (e.g., a sample of *Mycobacterium tuberculosis* itself). If the profile of the set of mycolic acids in the test sample is comparable to the profile of the set of the mycolic acids in the positive sample, that is indicative of the presence of *Mycobacterium tuberculosis* and thus of active tuberculosis infection. A
25 profile that is "comparable" to another is a profile that shows the same characteristic transitions specific for the majority (including, for example, all) of the mycolic acids in the set of mycolic acids.

The methods described above can be used to assess efficacy of treatment for active tuberculosis infection in an individual. "Treatment," as used herein, refers to
30 administration of a drug or other therapy in a dosage and for a length of time intended to eliminate infection with *Mycobacterium tuberculosis*. A test sample taken from an infected individual (an individual known to be infected with

Mycobacterium tuberculosis) prior to treatment can be assessed, and the profile of mycolic acids (e.g., of a set of C26- α -mycolic acids) can be compared to the profile of those mycolic acids in a test sample taken from the same infected individual after treatment. If the profiles remain comparable, that is indicative that the treatment lacks efficacy or that continued treatment is necessary, as the presence of the same profile of mycolic acids is indicative of the continued presence of *Mycobacterium tuberculosis*. Alternatively, a test sample taken from an infected individual after treatment can be assessed, and the profile of mycolic acids can be compared to the profile of mycolic acids in a positive sample as described above. An absence of a comparable profile of mycolic acids in the test sample as compared to the profile of mycolic acids in the positive sample indicates that the treatment is efficacious, as the absence of the sample profile is indicative of the absence of detectable *Mycobacterium tuberculosis*. Conversely, the presence of a comparable profile of mycolic acids in the test sample as compared to the profile of mycolic acids in the positive sample indicates the continued presence of *Mycobacterium tuberculosis*, and is indicative of a lack of efficacy of the treatment or of a need for continued treatment.

It is noted that although the description herein pertains to infection with *Mycobacterium tuberculosis*, particularly in human individuals, that the methods are also applicable to other forms of tuberculosis, such as *Mycobacterium bovis* in cattle. Profiles can be generated for the pathogen of interest, and the methods performed accordingly.

Exemplification

Materials and methods

Growth conditions. All strains were grown in Middlebrook medium (Difco Laboratories Ltd., West Molesey, UK), supplemented with 0.05% Tween 80 (v/v) (Sigma) either on 7H10 agar slants or in 7H9 broth, at 37°C; *M. ulcerans* was grown at 30°C. *Mycobacterium bovis* BCG Pasteur (ATCC 35734) and *M. tuberculosis* strains (H37Ra, CDC1551, HN878, and W4) were cultivated in broth under rotating

conditions (145 rpm) until the mid-logarithmic phase. Effects of different growth conditions on MA profiles were evaluated by cultivating *M. bovis* BCG on agar as well as in Dubos media using the Wayne model of hypoxic dormancy and were found to be insignificant (Shui *et al. J. Lipid Res* 48:1976-1984 (2007)). The non-tuberculous mycobacteria (NTM) strains MAC Q14, MAC M151, *M. goodii*, *M. fortuitum*, *M. simiae*, *M. kansasii*, *M. abscessus*, *M. chelonae*, and *M. xenopi* were grown on agar and kindly contributed by the Department of Microbiology at National University Hospital, Singapore. *Corynebacteria*, *Nocardia*, and all other mycobacterial species were cultivated as surface pellicles in broth and obtained from the laboratory of mamadou Dafe and Marie Laneelle. NTM isolates-1 and -2 were obtained from expectorated sputum specimens (see below), grown on agar, and provided as a kind gift by Kenneth Olivier (NIH, USA).

Animal experiments. All animal work was approved by the NITD Animal Welfare Committee (IUCAC 08-2008) and performed in compliance with national laws and institutional policies. Five-week-old female Balb/C mice were divided into three groups of ten animals each. Two groups were infected by intranasal inoculation with 10^2 to 10^3 colony forming units (CFU) of *M. tuberculosis* Beijing W4. One of these groups was treated with rifampicin daily (30 mg/kg body weight, a dose that is curative and far below the LD50 (Jayaram *et al., Antimicrob Agents Chemother* 47:2118-2124 (2003)). The second group was treated with vehicle only (PBS containing 0.25% carboxymethyl cellulose), and the third group was maintained as an uninfected, vehicle-treated control group. Five weeks post-infection, all mice were terminally bled under anesthesia *via* retro-orbital sinus puncture. Lungs were homogenized in PBS containing 1% triton X-100, and dilutions were plated for CFU analysis.

Clinical and Demographic Data of Participants. The 110 participants analyzed in this study represent at least four ethnic backgrounds originating from different geographical areas: the FIND study sites in the high-TB incidence countries of Vietnam (n=82), South Africa (n=2), and Uganda (n=16), and the NIH study site in the mid-TB incidence region of South Korea (n=10) at the National Masan Tuberculosis Hospital. All FIND samples were acquired under local IRB approvals and informed consent was obtained from all subjects. The Korean samples were

collected under an IRB-approved clinical protocol to study multidrug resistant *M. tuberculosis* (NCT00341601). Informed consent was obtained from all participants. From both sites, individuals were at least 18 years old (average 36), and the male-to-female ratio was 1:0.4.6.

- 5 Inclusion for enrollment was based on the presence of one or more clinical symptoms of pulmonary TB (persistent cough for at least three weeks, abnormal chest radiography, fever, night sweats, weight loss, or contact with an active TB case). Suspicion of TB was verified by positive acid fast bacillus (AFB) staining, solid culture and liquid culture using two consecutive sputum samples. For culture-
10 positive samples, speciation of the causative agent as MTB complex was performed using the Capilia method (Tauns Laboratories Inc., Japan). All individuals were tested for HIV, and positive cases were serologically confirmed. Participants from the FIND study sites had been without anti-TB treatment for at least 60 days prior to enrollment, whereas eight individuals from the Masan hospital were on anti-TB
15 chemotherapy at the time of specimen collection. All sputum samples were blinded for MA extraction and analysis. Sputum was further collected from two smear-positive patients with non-tuberculous mycobacteria (NTM) infections at the NIH clinical Center in the US under an IRB-approved NIAID clinical protocol studying atypical mycobacterial disease (NCT00018044).

- 20
Sputum Processing. No sputum specimen was concentrated. Expecterated sputum specimens (0.2-5 mL) from NIH study sites were digested and decontaminated by adding an equal volume of Sputasol solution (Oxoid Ltd., Basingstoke, UK), incubated at room temperature for 20 min with occasional vortexing, divided into
25 aliquots, and stored at -80°C until MA isolation. Sputum specimen from FIND study sites for MA extraction were neither digested nor decontaminated, but only homogenized by vortexing with glass beads for 1 min, divided into 0.5 mL aliquots, and stored at -70°C until MA isolation.

- For microscopy and culture using the MGIT system, sputum was
30 decontaminated using NALC-NaOH. Staining for all smears was standard Ziehl-Nielsen (=acid-fast blue) staining. The smears were examined under bright field microscopy at a 400x magnification.

Isolation of MAs from Bacteria, Human Sputum, and Mouse Lung Tissue. For MA isolation, bacteria, human sputum, and mouse lung homogenates were treated with chloroform/methanol (2:1, v/v) and shaken overnight at 4°C to inactivate the pathogens and extract free lipids. After separating the phases with water and centrifuging at 9000 rpm for 2 min, the lower organic phase (containing free lipids) was removed. To reduce ion suppression by phospholipids and hydrolyzed fatty acids, a second delipidation was carried out by repeating these steps. The upper aqueous phase was carefully removed, and the lower organic phase was collected and pooled with that from the first extraction. The intermediate layer was transferred to a fresh tube and dried. One volume of freshly prepared 20% tetrabutylammonium hydroxide was added to the dried material to hydrolyze the covalently linked MAs. The sample was vortexed thoroughly and heated for saponification in an oven at 105°C for 2 hrs. After cooling and acidification by adding ~ 50 µL of 12M HCl (desired pH ~4-5), 2 mL of hexane were added. The sample was vortexed vigorously for 1 min and spun at 5000 rpm for 2 min. The upper hexane layer was transferred to a fresh screw-cap tube, and the extraction was repeated twice with an additional 2 mL of hexane each. The hexane layer from the re-extraction was pooled with that from the first extraction and dried using a stream of N₂ gas.

HPLC/ESI/MS and Tandem ESI Analysis of MAs. HPLC/ESI/MS analysis of MAs was carried out as described (Shui *et al.*, *J. Lipid Res.* 48:1976-1984 (2007)). Briefly, a Waters XTerra column (1 mm x 150 mm) was used to separate lipids. Chloroform/methanol (1:1, v/v) with 5% of 300 mM piperidine (final concentration 15 mM) was used as the mobile phase for isocratic elution. The mass spectra were acquired from m/z 400 to 1500 on a Waters Micromass QTOF. Typically, 2 µL of sample were injected for analysis. MA profiles were obtained by combining QTOF MS spectra during the MA elution period during column separation, as described previously (Shui *et al.*, *J. Lipid Res.* 48:1976-1984 (2007)). Tandem MS of peaks from HHPLC/ESI/MS for all 33 stains analyzed was carried out with collision energies ranging from 40 to 80 volts.

Multiple Reaction Monitoring (MRM)-based MS Analysis of MAs. An Applied Biosystems Triple Quadrupole/Ion Trap mass spectrometer (4000Qtrap, foster City, California, USA) was used for quantification of individual MAs. Extracted MAs were resuspended in chloroform/methanol/200 mM piperidine (1:1:0.1, v/v/v) and
5 infused into the mass spectrometer with the same solution as the mobile phase at a flow rate of 8 μ L/min and measured in the negative ESI mode. Based on tandem MS of ions obtained from HPLC/ESI/MS and precursor ion scans of fatty acyl head groups, comprehensive sets of Multiple Reaction Monitoring (MRM) transitions were set up for quantitative analysis of MAs. The signal intensity of each MRM
10 value was normalized to the total MA counts or an internal standard for quantitative comparisons. A minimum number of 10 MRM transitions were acquired to obtain the averaged intensity. MRM transitions for mycobacterial mycolic acids were separated into two individual methods with collision energies ranging from 75 to 85 volts and 10 s of dwell time for each MRM transition. MRM transitions for
15 nocardiomycolic acids were separated into three individual methods with collision energies ranging from 60 to 70 volts and 10 s of dwell time for each MRM transition. MRM transitions for corynomycolic acids were run in a single method with collision energies ranging from 35 to 42 volts and 10 s of dwell time for each MRM transition.

20
Statistical Analysis. Concentrations of MAs extracted from human sputum and mouse lung tissues from different disease states were compared statistically. MA measurements were found to be distributed non-normally when employing a Shapiro-Wilk test. Therefore, the nonparametric Mann-Whitney U test was used to
25 compare TB-infected individuals with non-TB controls, and the Dunn's *post-hoc* test was used to compare TB-infected, rifampicin-treated mice and uninfected mice. Statistical analysis was carried out using GraphPad Prism.

Isotopic Correction. Due to the overlapping nature of pseudo-molecular ion peaks
30 of the species of interest with the M+2 isotope from another species that has a 2 Da lower mass, isotopic correction was required (Han, X. and Gross, R.W., *Mass Spectrom Rev.* 24:367-412 (2005)). Consider any MRM transition T_{MM_d} , where M

is the m/z of the parent and M_d is the m/z of the daughter. Let the peak intensity of this MRM transition be I_{M/M_d} . To perform isotopic correction, the correction factor Z must be calculated to obtain the corrected intensity $I_{M/M_d} = z \times I_{M/M_d}$. Assuming that m , the total number of carbons in the transition T_{M-2/M_d} , is much larger than 1
 5 (Han, X. and Gross, R.W., *Mass Spectrom Rev.* 24:367-412 (2005)), the correction factor Z is calculated as follows:

$$z \approx 1 - 6.05 \times 10^{-5} m^2 \left(\frac{I_{M-2}}{I_M} \right)$$

Clustering (Euclidean). Hierarchical clustering was used to group samples that were
 10 similar in MA profiles. The algorithm aims to group samples with the smallest Euclidean distances. If we consider each sample as an object, a Euclidean distance metric is calculated between each pair of objects. The clustering algorithm looks for the pair of objects that is separated by the shortest distance and groups them together to form a new object. The distances between such newly formed objects are then
 15 recalculated to form a new distance metric. These steps are performed iteratively until all objects are merged to become a single object. To display the results, a dendrogram consisting of U-shaped lines is drawn. The height of each U represents the distance between the objects. Hierarchical clustering was performed using the *pdist()* function provided in MatLab (R2006a, The MathWorks, Natick, MA, USA)
 20 with Euclidean distance metric.

RESULTS

Targeted Analysis of MAs Using Electrospray Ionization Mass Spectrometry (ESI/MS). We first used HPLC/ESI/MS (Shui *et al. J. Lipid Res* 48:1976-1984
 25 (2007)) to profile MAs in 33 CMN (*Corynebacterium-Mycobacterium-Nocardia*) organisms grown *n vitro* (see, for example, *Mycobacterium tuberculosis* Beijing strain in Fig. 1A). differences in MA profiles between genera were easily observed (Fig. 5), and allow for their identification. We next used tandem mass spectrometry (MS/MS) and collision-induced dissociation (CID) to further

characterize MAs in the 33 strains, revealing isobars with different lengths of alpha-branch fatty acids and of meromycolates (Fig. 1B). For example, fragmentation of m/z 1,164, which corresponds to an alpha-MA with a molecular composition of $C_{80}H_{156}O_3$ which is present in many species of mycobacteria including *M.*

5 *tuberculosis*, yielded product ions corresponding to alpha-branch fatty acids with different chain lengths: trace amounts of m/z 339 ($C_{22:0}$), and m/z 367 ($C_{24:0}$) and m/z 395 ($C_{26:0}$) as the predominant acyl species (Fig. 1B). Based on such product ion analysis, we established conditions for targeted analysis by multiple reaction monitoring (MRM). In MRM, a precursor ion of interest (e.g., m/z 1,108) is
10 selected in the first mass analyzer of a tandem mass spectrometer and fragmented in the collision cell, and a characteristic product ion (e.g., the acyl chain at m/z 367) is then selected in the second mass analyzer. For example, an alpha-MA with an m/z of 1,108 and a $C_{24:0}$ fatty acyl as the alpha-chain could thus be selectively monitored using the transition pair 1,108/367 (Fig. 1C). A total of 1,942 such MRM transitions
15 specific for individual MA species were defined (Table S1) and used to determine characteristic MA fingerprints of 33 CMN organisms (Fig. 1C). Synthetic C32 Ma (Laval *et al.*, *Anal. Chem.* 73:4537-4544 (2001)) with a MRM transition of 495/255 was used as an internal standard to determine limits of detection and to quantify individual levels of various alpha-, keto-, and methoxy-MAs extracted from mouse
20 lung tissue and human sputum samples. This MRM-based approach provides quantitative analysis that is very rapid (~2 min analytical time per sample, though this does not take into account sample preparation) and sensitive (limit of detection, LOD ~1 pg of mycolic acid extracts, an ~100-fold increase over existing methods (Shui *et al.* *J. Lipid Res* 48:1976-1984 (2007))).

25 In a next step, we investigated if mycolic acids can be extracted and analyzed directly from sputum samples from tuberculosis (TB) patients. While no mycolic acid signal could be measured in sputum from non-TB control patients, a number of mycolates were extracted from TB patient's sputum (Fig. 1D). Note that the lengths of MA extracted from bacterial cultures (Fig. 1A) differ from MA extracted
30 from sputum (Fig. 1D), with the major alpha-MA in culture being m/z 1,136, but m/z 1,164 in sputum. A similar trend of acyl lengthening can be observed for

methoxy-MA m/z 1,252 (culture) to m/z 1,280 (sputum) and keto-MA m/z 1,236 (culture) to m/z 1,264 (sputum) Fig. 1A, 1D, and data not shown).

We further determined the minimum number of bacterial cells necessary for our Ma detection approach, by performing a spiking experiment with a serial
5 dilution of *M. tuberculosis* cells added to non-TB sputum as well as culture medium. In sputum, around 10,000 cfu were sufficient to detect bacteria based on their MA signal (signal to noise ratio, $S/N=3$). A linear increase in MA levels was observed with increasing numbers of bacterial cells, for both sputum and medium. In extracts
10 from medium, MA signals were slightly higher than in extracts from sputum, which was probably due to MA extraction efficiency or ion suppression effects caused by the complex matrix of this body fluid (Fig. 1E).

Quantitative Analysis of MAs as a Diagnostic Marker for Tuberculosis Infection. Using a blinded format, we conducted a retrospective, multicenter, case-control
15 study of 70 patients with pulmonary tuberculosis (TB) with varying disease burdens and 40 non-TB controls (individuals with clinical symptoms of TB who were diagnosed as non-TB by culture). For both groups, the HIV status and bacterial burden were known (Fig. 2A). Strikingly, robust alpha-, keto-, and methoxy-MA signals were detected in as little as 200 μ L of sputum from TB patients (Fig. 2B).
20 This was too small of a sample volume for detecting MAs with alternative methods (Shui *et al. J. Lipid Res* 48:1976-1984 (2007)). Further, the concentration of major MAs detected in sputum obtained from TB-infected individuals was significantly (~100 times) higher than in samples from non-TB controls (Fig. 2C). As shown in Fig. 2E, we classified 66 out of the 70 TB patients correctly as TB positive ("true
25 positives") and 37 out of 40 non-TB controls correctly as non-TB ("true negatives"). Three were falsely classified as TP positive ("false positives") and four were falsely classified as non-TB ("false negatives"). Using these data, we calculated a statistical sensitivity of 94% and a specificity of 93% (sensitivity: number of true positives divided by the sum of true positives and false negatives; specificity: number of true
30 negatives divided by the sum of true negatives and false positives) and even slightly better values for the HIV positive individuals alone (Fig. 2F).

Careful inspection of the data obtained for each of the 110 participants showed that individual molecular species varied in their power to differentiate between non-TB controls and TB patients. Receiver operating characteristic (ROC) curves were computed to compare classifying characteristics of individual MA species (Fig. 2D). MAs with (i) C26 alpha-branches, rather than those with C24 and C22 branches (Fig. 2C, 2D, and data not shown), and (ii) alpha-MAs, rather than oxygenated MAs such as methoxy-MAs or keto-MAs (Fig. 2D, Fig. 4), were best suited for diagnostic purposes. Alpha-MA $C_{80}H_{156}O_3$ (m/z 1,164/395, Fig. 1B) provided the best accuracy in our study population, yielding an area under the ROC curve of 0.94 for classification as TB/non-TB (Fig. 2D). Thus, this sensitive targeted MS analysis allowed for robust detection of MAs from minimally processed, small-volume sputum extracts. The large number of MAs included in the list helped identify those MAs present in clinical samples.

We next evaluated the possibility of using the full profiles of mycobacterial MAs, rather than individual MAs such as C26 alpha-MA, for diagnostic classification. This approach was based on the assumption that MA profiles of bacterial species grown *in vitro* reflect those found in sputum extracts. We used Euclidean cluster analysis to group mycobacterial strains according to similarities in their MA profiles. Strikingly, all sputum profiles analyzed (four representative samples are shown in Fig. 14) fell within the *M. tuberculosis* (MTB) cluster. We further analyzed MAs from two clinical non-tuberculous mycobacterial (NTM) isolates (NTM isolate-1 and -2) and one corresponding sputum sample (NTM sputum-2). NTM isolate-1 was characterized as *M. avium* complex (MAC) by sequencing the 16S rRNA and *rpoB* regions (data not shown). Interestingly, the MA profile of NTM isolate-1 was closest to those of MAC M151 and MAC Q14 in our analysis. NTM isolate-2 was characterized as *M. massiliense*, a rapidly growing mycobacterium that is indistinguishable from *M. chelonae*/*M. abscessus* with partial 16S rRNA gene sequencing (Simmon *et al.*, *J. Clin. Microbiol.* 45:1978-1980 (2007)). MAs from both *M. chelonae* and *M. abscessus* were profiled in this study and were found to be most similar to NTM isolate-2 and the corresponding sputum (NTM sputum-2). Thus, in addition to indicating TB infection (Fig. 2), the above

results demonstrated that MA profiles allow for classification of the infecting mycobacteria (Fig. 14).

HIV co-infection is a complicating factor in TB diagnostics using assays that rely on immunological readouts, such as interferon-gamma release. However, an approach that directly monitors bacterial factors such as MAs, should not be influenced by such complications. We examined the effect of co-infection with HIV by comparing the predictive power of MA signals for both the complete set of 110 samples (Fig. 2E) and the 39 HIV-positive patients only (Fig. 2F). Sensitivities and specificities were very comparable, indicating that this approach is not impaired by HIV co-infection.

MAs as Markers for Drug Efficacy in a Controlled Laboratory Experiment. The majority of the study subjects (those from the FIND studies) had not received anti-TB chemotherapy for at least 60 days prior to enrollment. Eight of the ten Korean patients had received combination therapy with first- and second-line drugs for more than a month at the time of specimen collection, but remained sputum positive (two of these were MDR patients). We did not observe differences (either qualitative or quantitative) in MA profiles between individuals who received anti-TB chemotherapy and those who did not within this limited number of patients.

To evaluate our method for monitoring drug efficacy, we measured MAs in lung-tissue of mice infected with *M. tuberculosis*. These animals are routinely used in pre-clinical studies for development of novel treatments against TB. One commonly used marker for drug efficacy is body weight, which is only indirectly related to TB infection. The signatures of major MAs in mouse lung (Fig. 3A) were very comparable to those observed in human sputum samples (Fig. 2B). The bacillary burden of these mice as assessed by colony forming units (cfu) in their lungs was 15.2×10^6 on average (as assessed in 5 out of the 10 animals tested for MA levels). We also measured MA levels in TB-infected mice that had been treated with the bactericidal antibiotic rifampicin (30 mg/kg body weight for four weeks), after which the mice were cured of TB (no detectable cfu in the lungs) Jayaram *et al.*, *Antimicrob Agents Chemother.* 47:2118-2124 (2003)). Impressively, the MAs in lung tissue of treated mice were significantly reduced compared to untreated mice,

indicating that MAs were effectively cleared at some point during the four-week treatment (Fig. 3B). Thus, direct monitoring of pulmonary MAs can be used to differentiate TB-infected mice from healthy ones.

5 DISCUSSION

The experiments herein have identified a subclass of MAs extracted directly from patients' sputum and mouse lungs, which can be used as a diagnostic marker for acute TB infection. The MA signal clearly differentiates these active TB samples from non-active or cured TB, as no MA (apart from three false positive
10 samples) were detected in our non-TB control group (suspected TB patients) or in rifampicin-treated mice. Non-oxygenated MAs with C26 acyl moieties in their alpha-branch best classified active versus non-active TB. The rapid and direct measurement of distinct bacterial components described herein yields high sensitivity and selectivity in poorly defined heterogenous biological material
15 (sputum and lung tissue); furthermore, because no culturing was required, the method was quick and safe, and sample preparation was simple and required no chemical derivatization, thus simplifying sample preparation and handling. The approach was feasible for four ethnicities and varying disease burdens.

A comparison of the described method to the acid fast bacilli (AFB) smear
20 indicated that heavy burden cases (i.e., smear ++ and +++) were detected with equal accuracy by our method (100%; 15/15 smear ++ and 7/7 smear +++) . Almost identical detection (97.5%) was seen with smear + cases (39/40). In more difficult cases in which the smear results were unclear (smear scanty) or negative (smear negative/culture positive), the methods herein were able to identify positive TB
25 cases from $\frac{3}{4}$ (75%) of scanty smear results and $\frac{1}{4}$ (92.5%) smear negative/culture positive results (Fig. 2C), indicating that MA analysis is an important development leading to better detection of TB in particularly critical subset of cases which are typically released without treatment and thus pose a risk for disease dissemination.

The false negatives detected with this approach could be explained by limits
30 in extraction efficiency and instrument sensitivity. To evaluate further the power of this method in these borderline cases, we analyzed sputum samples from an additional 50 smear negative/culture positive subjects as replication cohort. These

subjects all produced two consecutive smear negative sputa, which were subsequently diagnosed as TB positive by liquid culture. Three of these 50 subjects (6%) were detected positively with the described method, indicating that the results are superior to AFB staining. Further adjustments to improve sensitivity may include enhancement of mycolic acid extraction efficiency (e.g., novel extraction approaches based on solid phases or supercritical fluid extraction) or increased analytical sensitivity (e.g., improved ionization sources and ion funnels).

The false-positive cases remained false positive after re-sampling and re-measuring banked specimens.

C26 alpha-MA was sufficient to differentiate between active and non-active TB. The inclusion of all mycobacterial MA added specificity to the approach and allowed for additional classification based on MA profiles (Fig. 14). Patients infected with non-tuberculous mycobacteria (NTM) could be clearly distinguished from those infected with TB using cluster analysis of MA patterns. Differentiation between MTB and NTM infection can be important because markedly different chemotherapeutic regimens are used to treat these infections. Current technologies that differentiate between these infections and provide species identification rely on culture and DNA amplification of species-specific sequences, leading to a delay of up to 6-8 weeks before information needed for patient care is obtained (Garcia-Quintanilla, A. *et al.*, *J. Clin. Microbiol.* 40:4646-4651 (2002); Shrestha, N.K. *et al.*, *J. Clin. Microbiol.* 41:5121-5126 (2003); Bruijnesteijn van Coppenract, E.S. *et al.*, *J. Clin. Microbiol.* 42:2644-2650 (2004); Marris, E., *Nat Med.* 13:267 (2007)).

The techniques described herein provide a powerful new approach to monitor subtle differences in MA structure that may be important for pathogenicity. Thus, the methods are applicable to basic chemistry and biology of MAs (e.g., biosynthesis and transport) and their immunology (e.g., CD1-mediated activation of T cells) as well. Furthermore, they will be useful for longitudinal testing of large numbers of specimens for drug efficacy and prediction of relapse. In addition, although the approach requires certain specialized equipment, such analysis can be feasible because of the increased technical capabilities of reference laboratories in areas with endemic TB infections. MAs are inert and stable during sampling, even at tropical temperatures and during storage for extended periods of time.

Importantly, direct detection of bacteria-derived molecules circumvents the need for indirect readouts from immunological tests, which may be difficult to interpret in patients with multiple infections and compromised immune functions (e.g., elderly or HIV positive patients). With up to 50% of HIV positive patients succumbing to TB in some geographic areas, it is crucial that diagnostic tools such as the one described herein accurately detect TB in both HIV negative and HIV positive individuals.

Table S2 shows clinical and demographic data of study participants. Individuals were classified as having pulmonary TB based on clinical examination, some with additional sites of MTB infection, or concomitant disease other than lung or heart disease, such as AIDS-defined illnesses and diabetes. For the replication cohort (smear negative, culture positive), results of the two consecutive liquid culture results are given.. Abbreviations: Prev. Tx, previous TB treatment; AB in past 8w, antibiotic treatment in past 8 weeks; N.A., not applicable; N.D., not determined; NK, now known; SS+, smear and culture positive; S-C+, smear negative, culture positive; TN, true negative; TP, true positive; Antibiotics: H, isoniazide; R, rifampicin; E, ethambutol; Z, pyrazinamide; S, streptomycin; K, kanamycin; P, p-aminosalicylic acid; T, prothionamide; C, cycloserine; Lf, levofloxacin; Mf, moxifloxacin.

Supplementary Table 2

Sample #	Origin	sex	age	prev. Tx	other diseases	AB past 3 w	appearance	chest radiography conclusion	cigarettes #/y	smear	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
TB positive, HIV negative																
1	Vietnam	F	26	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
2	Vietnam	M	39	No	No	Yes	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/20	1+	3+	Pos	MTB complex	Neg	SS+	TP
3	Vietnam	M	50	No	No	Yes	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/30	2+	3+	Pos	MTB complex	Neg	SS+	TP
4	Vietnam	M	32	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	4/10	1+	2+	Pos	MTB complex	Neg	SS+	TP
5	Vietnam	M	37	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	13/15	1+	2+	Pos	MTB complex	Neg	SS+	TP
7	Vietnam	M	37	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	3+	Pos	MTB complex	Neg	SS+	TP
8	Vietnam	M	22	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/3	1+	3+	Pos	MTB complex	Neg	SS+	TP
9	Vietnam	M	53	No	Diabetes	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	5/20	1+	2+	Pos	MTB complex	Neg	SS+	TP
10	Vietnam	M	37	No	Other	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	2+	2+	Pos	MTB complex	Neg	SS+	TP
11	Vietnam	F	25	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
12	Vietnam	F	29	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	3+	Pos	MTB complex	Neg	SS+	TP
13	Vietnam	F	36	No	No	No	Gravely ill	TB likely (pulmonary, pleural, pericardial)	No	2+	3+	Pos	MTB complex	Neg	SS+	TP
14	Vietnam	M	36	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
15	Vietnam	M	19	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	3+	Pos	MTB complex	Neg	SS+	TP
16	Vietnam	M	50	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	30/30	1+	1+	Pos	MTB complex	Neg	SS+	TP
17	Vietnam	F	33	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	3+	Pos	MTB complex	Neg	SS+	TP
18	Vietnam	F	30	No	AIDS	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	2+	3+	Pos	MTB complex	Pos	SS+	TP
19	Vietnam	M	25	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
20	Vietnam	M	33	No	No	No	Mildly ill	Other	10/10	2+	3+	Pos	MTB complex	Neg	SS+	TP
21	Vietnam	M	44	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/10	1+	<20	Pos	MTB complex	Neg	SS+	TP
22	Vietnam	F	51	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	3+	3+	Pos	MTB complex	Neg	SS+	TP
23	Vietnam	F	19	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	2+	2+	Pos	MTB complex	Neg	SS+	TP
24	Vietnam	M	29	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	6/10	2+	1+	Pos	MTB complex	Neg	SS+	TP

Sample #	Origin	Sex	Age	prev. Tx	other diseases	AS past 8 w	appearance	chest radiography conclusion	cigarettes #/y	sneez	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
25	Vietnam	M	42	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/20	1+	1+	Pos	MTB complex	Neg	SS+	TP
26	Vietnam	M	35	No	Diabetes	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
27	Vietnam	M	18	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	40/10	2+	3+	Pos	MTB complex	Neg	SS+	TP
28	Vietnam	M	41	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/20	2+	1+	Pos	MTB complex	Neg	SS+	TP
29	Vietnam	M	60	No	No	No	Gravely ill	TB likely (pulmonary, pleural, pericardial)	No	1+	2+	Pos	MTB complex	Neg	SS+	TP
30	Vietnam	F	52	No	Diabetes	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	2+	2+	Pos	MTB complex	Neg	SS+	TP
31	Vietnam	F	21	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	2+	3+	Pos	MTB complex	Neg	SS+	TP
32	Vietnam	M	30	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/10	2+	3+	Pos	MTB complex	Neg	SS+	TP
34	Vietnam	M	43	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	40/20	1+	3+	Pos	MTB complex	Neg	SS+	TP
35	Vietnam	F	18	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	1+	3+	Pos	MTB complex	Neg	SS+	TP
36	Vietnam	F	19	No	No	No	Gravely ill	TB likely (pulmonary, pleural, pericardial)	No	3+	3+	Pos	MTB complex	Neg	SS+	TP
37	Vietnam	M	33	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	7/10	2+	3+	Pos	MTB complex	Neg	SS+	TP
42	Vietnam	M	22	No	Other	NK	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	2+	Pos	MTB complex	Neg	S-C+	TP
44	Vietnam	M	52	No	No	NK	Gravely ill	Pneumonia or atypical TB	10/30	Neg	<20	Pos	MTB complex	Neg	S-C+	FN
45	Vietnam	M	41	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	3/15	Neg	<20	Pos	MTB complex	Neg	S-C+	FN
46	Vietnam	F	21	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Scanty	1+	Pos	MTB complex	Neg	S-C+	FN
47	Vietnam	M	45	No	No	No	Gravely ill	Pneumonia or atypical TB	30/6	Neg	1+	Pos	MTB complex	Neg	S-C+	FN
101	S Korea	M	33	5	No	EPTCLIS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	3+	3+	N.D.	N.D.	Neg	SS+	TP
102	S Korea	M	38	3	No	No	NK	TB likely (pulmonary, pleural, pericardial)	Yes	3+	3+	N.D.	N.D.	Neg	SS+	TP
103	S Korea	M	38	5	Diabetes	No	NK	TB likely (pulmonary, pleural, pericardial)	Yes	3+	3+	N.D.	N.D.	Neg	SS+	TP
104	S Korea	M	44	Yes	NK	HERZS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	Scanty	N.D.	N.D.	Neg	SS+	TP
105	S Korea	M	26	No	NK	HERS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	Neg	N.D.	N.D.	Neg	SS+	TP
106	S Korea	M	40	No	NK	HERS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	1+	N.D.	N.D.	Neg	SS+	TP
107	S Korea	M	45	No	Diabetes	HERZS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	1+	N.D.	N.D.	Neg	SS+	TP
108	S Korea	M	51	Yes	NK	HERZS	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	Scanty	N.D.	N.D.	Neg	SS+	TP

Sample #	Origin	Sex	Age	prev. Tx	other diseases	AB past 6 w	appearance	chest radiography conclusion	cigarettes #/y	smear	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
109	S Korea	M	NK	NK	NK	SPTCMf	NK	TB likely (pulmonary, pleural, pericardial)	Yes	1+	Scanty	N.D.	N.D.	Neg	SS+	TP
110	S Korea	M	74	No	NK	ZKPLf	NK	TB likely (pulmonary, pleural, pericardial)	Yes	Scanty 1+		N.D.	N.D.	Neg	SS+	TP
TB Positive, HIV positive																
40	Vietnam	M	32	No	AIDS	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	10/15	1+	3+	Pos	MTB complex	Pos	SS+	TP
41	Vietnam	M	24	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/8	1+	3+	Pos	MTB complex	Pos	SS+	TP
43	Vietnam	M	27	No	AIDS	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	10/10	2+	3+	Pos	MTB complex	Pos	SS+	TP
48	Vietnam	M	35	Yes	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	3+	Pos	MTB complex	Pos	SS+	TP
49	Vietnam	M	37	No	AIDS	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	3+	3+	Pos	MTB complex	Pos	SS+	TP
50	Vietnam	M	30	No	No	No	Gravely ill	TB likely (pulmonary, pleural, pericardial)	3/15	1+	3+	Pos	MTB complex	Pos	SS+	TP
51	Vietnam	M	35	No	AIDS	NK	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/6	Scanty 2+		Pos	MTB complex	Pos	SS+	TP
56	Vietnam	M	42	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	2+	Pos	MTB complex	Pos	SS+	TP
58	Vietnam	M	35	No	Other	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	15/20	1+	1+	Pos	MTB complex	Pos	SS+	TP
59	Vietnam	M	32	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	2+	2+	Pos	MTB complex	Pos	SS+	TP
60	Vietnam	M	29	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	40/10	1+	2+	Pos	MTB complex	Pos	SS+	TP
61	Vietnam	M	37	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	1+	Pos	MTB complex	Pos	SS+	TP
62	Vietnam	M	43	Yes	No	Cefotaxim	Moderately ill	TB likely (pulmonary, pleural, pericardial)	15/20	3+	3+	Pos	MTB complex	Pos	SS+	TP
63	Vietnam	M	36	No	AIDS	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	1+	1+	Pos	MTB complex	Pos	SS+	TP
64	Vietnam	F	35	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	1+	3+	Pos	MTB complex	Pos	SS+	FN
68	Vietnam	F	37	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	2+	Neg	MTB complex	Pos	SS+	TP
76	Vietnam	M	33	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/10	1+	Neg	Neg	N.D.	Pos	SS+	TP
81	Vietnam	M	27	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	10/10	1+	3+	Pos	MTB complex	Pos	SS+	TP
83	Vietnam	M	25	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	5/10	1+	1+	Neg	MTB complex	Pos	SS+	TP
84	Vietnam	M	24	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	3/5	Scanty 2+		Pos	MTB complex	Pos	SS+	TP

Sample #	Origin	sex	age	prev. Tx	other diseases	AB past 6 w	appearance	chest radiography conclusion	cigarettes #/y	smear	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
TB negative, HIV negative																
86	Uganda	M	34	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Neg	Non-TB	TN
8	Vietnam	M	20	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
53	Vietnam	M	41	No	No	No	Moderately ill	Pneumonia or atypical TB	10/10	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
54	Vietnam	M	39	No	No	No	Moderately ill	Pneumonia (TB unlikely)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	FP
55	Vietnam	F	23	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
67	Vietnam	F	20	No	No	No	Mildly ill	Pneumonia (TB unlikely)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	FP
65	Vietnam	M	41	No	No	NK	Moderately ill	TB likely (pulmonary, pleural, pericardial)	15/20	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
66	Vietnam	M	40	No	No	NK	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
67	Vietnam	F	64	Yes	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
69	Vietnam	F	20	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
70	Vietnam	M	46	Yes	Diabetes	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
71	Vietnam	M	42	Yes	Diabetes	Cephalexin	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/30	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
72	Vietnam	M	25	No	No	NK	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
73	Vietnam	F	65	No	No	Cefixim	Mildly ill	TB likely (pulmonary, pleural, pericardial)	5/30	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
74	Vietnam	M	74	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/30	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
75	Vietnam	M	34	No	No	NK	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
77	Vietnam	M	55	No	Other	Cefpodoxim	Moderately ill	TB likely (pulmonary, pleural, pericardial)	40/30	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
78	Vietnam	M	63	No	No	NK	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
79	Vietnam	M	44	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	5/15	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
80	Vietnam	M	40	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
82	Vietnam	M	19	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg	N.A.	Neg	Non-TB	TN
TB negative, HIV positive																
85	Uganda	F	30	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
87	Uganda	F	55	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
88	Uganda	M	33	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
89	Uganda	F	43	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
90	Uganda	F	22	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
91	Uganda	F	47	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
92	Uganda	F	39	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN

Sample #	Origin	sex	age	prev. Tx	other diseases	AB past 6 w	appearance	chest radiography conclusion	cigarettes #/yr	smear	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
93	Uganda	M	34	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
94	Uganda	F	28	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
95	Uganda	F	35	NK	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
96	Uganda	F	27	No	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
97	Uganda	M	34	No	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
98	Uganda	M	33	No	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
99	Uganda	F	31	No	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
100	Uganda	F	19	Yes	NK	No	chest pain, fever	TB unlikely	No	Neg	Neg	N.D.	N.A.	Pos	Non-TB	TN
38	S Africa	F	30	No	AIDS	No	Not ill	Pneumonia (TB unlikely)	No	Neg	Neg	Neg	N.A.	Pos	Non-TB	TN
52	S Africa	M	35	Yes	AIDS	Yes	Mildly ill	Pneumonia (TB unlikely)	Not known	Neg	Neg	Neg	N.A.	Pos	Non-TB	TN
52	Vietnam	F	29	No	AIDS	NK	Moderately ill	Pneumonia or atypical TB	No	Neg	Neg	Neg	N.A.	Pos	Non-TB	FP
33	Vietnam	F	25	No	Heart disease	No	Moderately ill	Pneumonia or atypical TB	No	Neg	Neg	Neg	N.A.	Pos	Non-TB	TN
replication cohort (smear negative, culture positive)																
111	Vietnam	F	34	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Pos/Neg	MTB complex	Neg	S-C+	TP
112	Vietnam	F	23	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	<20	Pos/NK	MTB complex	Neg	S-C+	FN
113	Vietnam	M	41	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	30/20	Neg	Neg	Pos/Neg	MTB complex	Neg	S-C+	FN
114	Vietnam	M	34	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/5	Neg	Neg	Pos/Neg	MTB complex	Neg	S-C+	TP
115	Vietnam	F	23	No	No	No	Mildly ill	Pneumonia or atypical TB	No	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
116	Vietnam	M	33	No	No	No	Gravely ill	Pneumonia or atypical TB	20/7	Neg	Neg	Pos/Neg	MTB complex	Neg	S-C+	FN
117	Vietnam	M	23	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Scanty	Neg	Neg/Pos	MTB complex	Neg	S-C+	FN
118	Vietnam	M	63	No	No	No	Mildly ill	Other	20/45	Neg	Pos	Pos/Neg	MTB complex	Neg	S-C+	FN
119	Vietnam	M	38	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/10	Neg	2+	Pos/Pos	MTB complex	Neg	S-C+	FN
120	Vietnam	F	23	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Pos	Pos/Neg	MTB complex	Neg	S-C+	FN
121	Vietnam	M	57	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/20	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
122	Vietnam	M	48	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	10/4	Neg	Neg	Pos/Neg	MTB complex	Neg	S-C+	FN
123	Vietnam	M	34	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Scanty	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
124	Vietnam	M	36	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	9/6	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
125	Vietnam	M	24	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
126	Vietnam	M	28	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg/Pos	MTB complex	Neg	S-C+	FN
127	Vietnam	F	31	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Pos	Pos/Neg	MTB complex	Neg	S-C+	FN

Sample #	Origin	sex	age	prev. Tx	other diseases	AB past 6 w	appearance	chest radiography conclusion	diagnosis #/y	small	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
128	Vietnam	M	47	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	TP
129	Vietnam	M	52	No	No	No	Mildly ill	Other	7/10	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
130	Vietnam	M	46	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	5/17	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
131	Vietnam	M	43	No	Other	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/20	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
132	Vietnam	M	59	No	Other	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/50	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
133	Vietnam	M	37	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
134	Vietnam	M	56	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/10	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
135	Vietnam	M	35	No	No	No	Moderately ill	Pneumonia or atypical TB	10/13	Neg	1+	Neg/Pos	MTB complex	Neg	S-C+	FN
136	Vietnam	M	41	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	5/12	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
137	Vietnam	M	25	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
138	Vietnam	M	54	No	bronchiectasis	No	Mildly ill	Pneumonia or atypical TB	40/25	Neg	Neg	Pos/Pos	MTB complex	Neg	S-C+	FN
139	Vietnam	M	26	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/6	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
140	Vietnam	M	46	No	No	No	Moderately ill	Pneumonia or atypical TB	5/17	Neg	Neg	Pos/Pos	MTB complex	Neg	S-C+	FN
141	Vietnam	M	67	NK	No	No	Moderately ill	Pneumonia or atypical TB	No	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
142	Vietnam	F	36	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
143	Vietnam	F	46	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
144	Vietnam	M	32	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	1+	Pos/Pos	MTB complex	Neg	S-C+	FN
145	Vietnam	M	46	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/10	Neg	<20	Pos/Pos	MTB complex	Neg	S-C+	FN
146	S Africa	M	37	NK	AIDS	No	Mildly ill	no x ray performed	NK	Neg	1+	Pos/Pos	MTB complex	Pos	S-C+	FN
147	S Africa	M	32	No	No	No	Moderately ill	Pneumonia or atypical TB	No	Neg	Neg	Pos/Pos	MTB complex	Pos	S-C+	FN
148	S Africa	M	46	No	No	No	Mildly ill	no x ray performed	No	Neg	Neg	Pos/Pos	MTB complex	Pos	S-C+	FN
149	S Africa	F	46	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Neg/Pos	MTB complex	Pos	S-C+	FN
150	S Africa	F	30	Yes	No	No	Mildly ill	healthy	No	Neg	Pos	Pos/Pos	MTB complex	Pos	S-C+	FN
151	S Africa	F	32	Yes	AIDS	No	Mildly ill	healthy	No	Neg	Neg	Pos/Pos	MTB complex	Pos	S-C+	FN
152	S Africa	F	50	No	AIDS	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	No	Neg	Neg	Pos/Pos	MTB complex	Pos	S-C+	FN
153	S Africa	M	46	No	No	No	Mildly ill	no x ray performed	No	Neg	Neg	Pos/Pos	MTB complex	Pos	S-C+	FN
154	Vietnam	M	38	No	Other	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/20	Neg	1+	Pos/Pos	MTB complex	Pos	S-C+	FN
155	Vietnam	M	50	No	No	No	Mildly ill	TB likely (pulmonary, pleural, pericardial)	20/50	Neg	1+	Pos/Pos	MTB complex	Pos	S-C+	FN
156	Vietnam	M	43	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/50	Neg	1+	NK/Pos	MTB complex	Pos	S-C+	FN

Sample #	Origin	sex	age	prev. Tx	other diseases	AB post b w	appearance	chest radiography conclusion	cigarettes #/y	smear	solid culture	liquid culture	speciation	HIV status	diagnosis	MA detection
157	Vietnam	M	41	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	2/20	Neg	1+	Neg/Pos	MTB complex	Pos	SC+	FN
158	Vietnam	M	28	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	3/10	Neg	<20	Neg/Pos	MTB complex	Pos	SC+	FN
159	Vietnam	M	55	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	20/30	Neg	<20	Neg/Pos	MTB complex	Pos	SC+	FN
160	Vietnam	M	32	No	No	No	Moderately ill	TB likely (pulmonary, pleural, pericardial)	10/15	Neg	<20	Pos/Pos	MTB complex	Pos	SC+	FN

The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without
5 departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A method of assessing a test sample for the presence of *Mycobacterium tuberculosis*, comprising preparing a profile of mycolic acids in the test sample using electrospray ionization mass spectrometry, and identifying the presence of C26- α -mycolic acids based on the profile, wherein the presence of C26- α -mycolic acids is indicative of the presence of *Mycobacterium tuberculosis*.
2. A method of assessing a test sample for the presence of *Mycobacterium tuberculosis*, comprising preparing a profile of a set of mycolic acids in the test sample using electrospray ionization mass spectrometry, and comparing the profile of the set of mycolic acids in the test sample to a profile of the set of mycolic acids in a positive sample prepared using electrospray ionization mass spectrometry, wherein the presence of a comparable profile of mycolic acids in the test sample and in the positive sample is indicative of the presence of *Mycobacterium tuberculosis*.
3. The method of Claim 2, wherein the set of mycolic acids comprises mycolic acids selected from the group of mycolic acids set forth in Supplementary Table 1.
4. The method of Claim 3, wherein the set of mycolic acids comprises C26- α -mycolic acids.
5. The method of Claim 3, wherein the set of mycolic acids comprises C24- α -mycolic acids.
6. The method of Claim 3, wherein the set of mycolic acids comprises C22- α -mycolic acids.

7. The method of Claim 3, wherein the set of mycolic acids comprises all of the mycolic acids set forth in Supplementary Table 1.
- 5 8. A method of diagnosing an active tuberculosis infection in an individual, comprising preparing a profile of mycolic acids in a test sample from the individual using electrospray ionization mass spectrometry, and identifying the presence of C26- α -mycolic acids based on the profile, wherein the presence of C26- α -mycolic acids is diagnostic for an active tuberculosis
10 infection in the individual.
9. A method of diagnosing an active tuberculosis infection in an individual, comprising preparing a profile of a set of mycolic acids in a test sample from the individual using electrospray ionization mass spectrometry, and
15 comparing the profile of the set of mycolic acids in the test sample to a profile of the set of mycolic acids in a positive sample prepared using electrospray ionization mass spectrometry, wherein the presence of a comparable profile of mycolic acids in the test sample and in the positive sample is diagnostic for an active tuberculosis infection in the individual.
20
10. The method of Claim 9, wherein the set of mycolic acids comprises mycolic acids selected from the group of mycolic acids set forth in Supplementary Table 1.
- 25 11. The method of Claim 10, wherein the set of mycolic acids comprises C26- α -mycolic acids.
12. The method of Claim 10, wherein the set of mycolic acids comprises C24- α -mycolic acids.
30
13. The method of Claim 10, wherein the set of mycolic acids comprises C22- α -mycolic acids.

14. The method of Claim 10, wherein the set of mycolic acids comprises all of the mycolic acids set forth in Supplementary Table 1.
- 5 15. A method of assessing efficacy of a treatment for active tuberculosis infection in an individual, comprising preparing a profile of a set of mycolic acids in a test sample from the individual before the treatment using electrospray ionization mass spectrometry, and comparing the profile of the set of mycolic acids in the test sample to a profile of the set of mycolic acids in a test sample from the individual after the treatment using electrospray ionization mass spectrometry, wherein the absence of a comparable profile of mycolic acids in the test sample before treatment and in the test sample after treatment is indicative of efficacy of the treatment, and the presence of a comparable profile of mycolic acids in the test sample before treatment and in the test sample after treatment is indicative of a lack of efficacy of the treatment.
- 10 16. A method of assessing efficacy of a treatment for active tuberculosis infection in an individual, comprising preparing a profile of a set of mycolic acids in a test sample from the individual after the treatment using electrospray ionization mass spectrometry, and comparing the profile of the set of mycolic acids in the test sample to a profile of the set of mycolic acids in a positive sample prepared using electrospray ionization mass spectrometry, wherein the absence of a comparable profile of mycolic acids in the test sample and in the positive sample is indicative of efficacy of the treatment, and the presence of a comparable profile of mycolic acids in the test sample and in the positive sample is indicative of a lack of efficacy of the treatment.
- 15 17. The method of Claim 16, wherein the set of mycolic acids comprises mycolic acids selected from the group of mycolic acids set forth in Supplementary Table 1.
- 20
- 25
- 30

18. The method of Claim 17, wherein the set of mycolic acids comprises C26- α -mycolic acids.
- 5 19. The method of Claim 17, wherein the set of mycolic acids comprises C24- α -mycolic acids.
20. The method of Claim 17, wherein the set of mycolic acids comprises C22- α -mycolic acids.
- 10 21. The method of Claim 17, wherein the set of mycolic acids comprises all of the mycolic acids set forth in Supplementary Table 1.

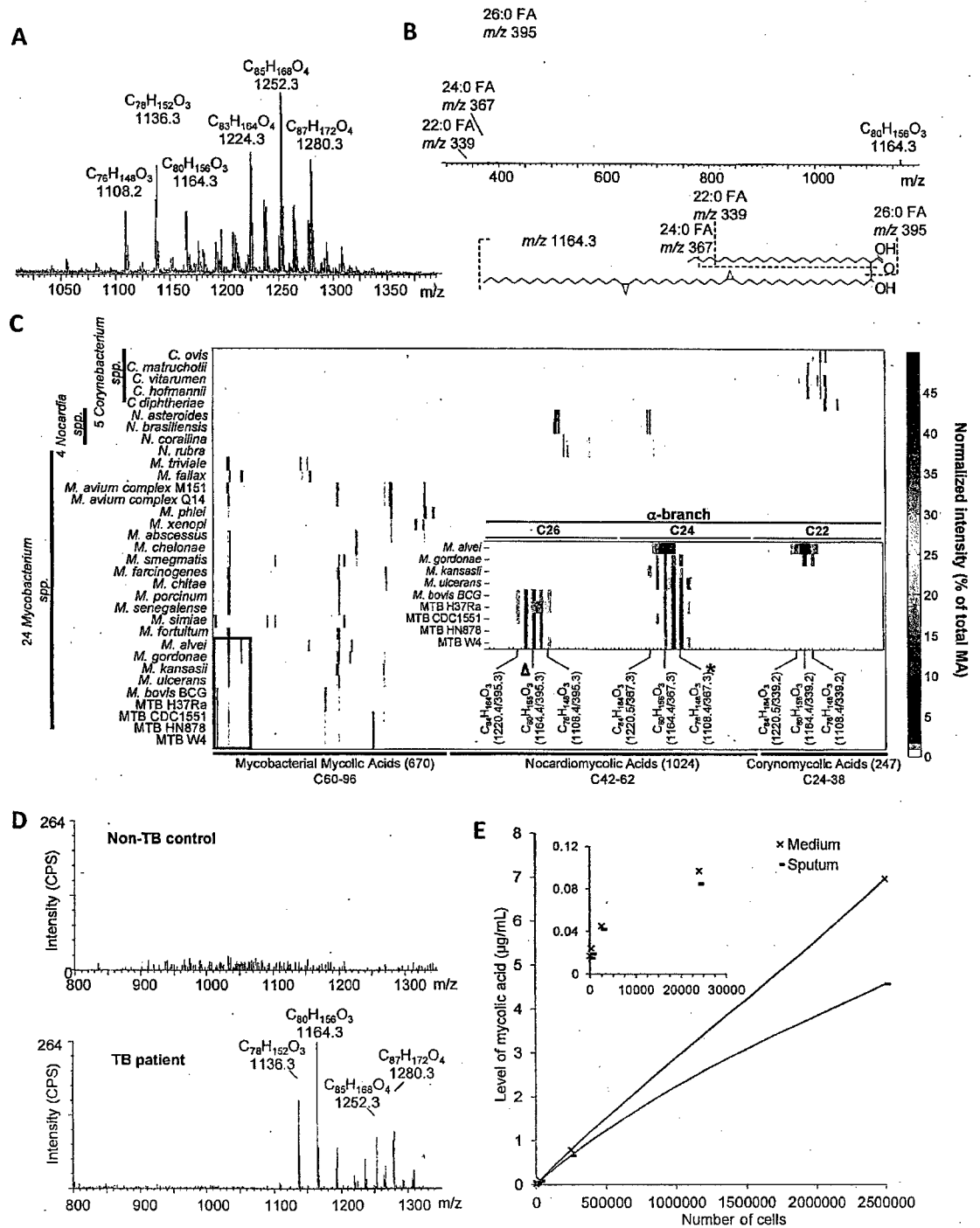


FIG. 1

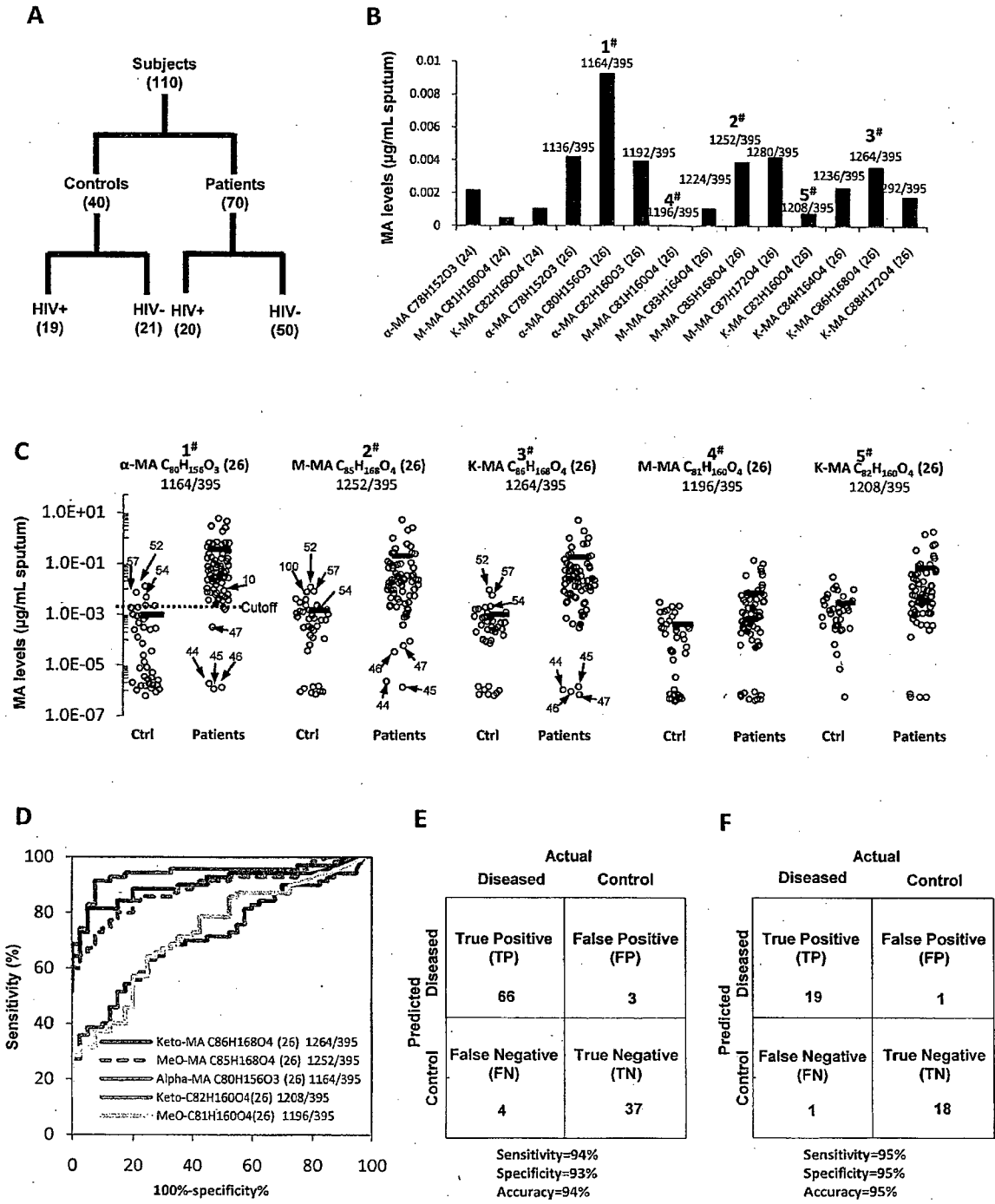


FIG. 2

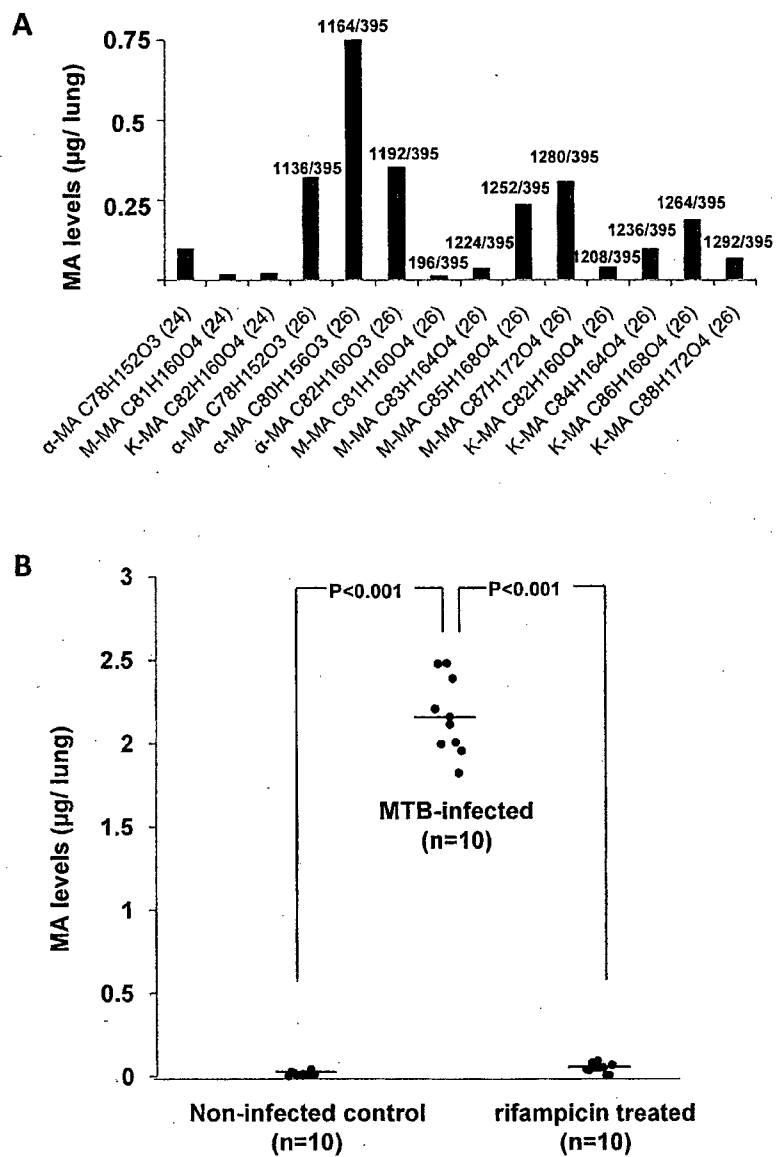


FIG. 3

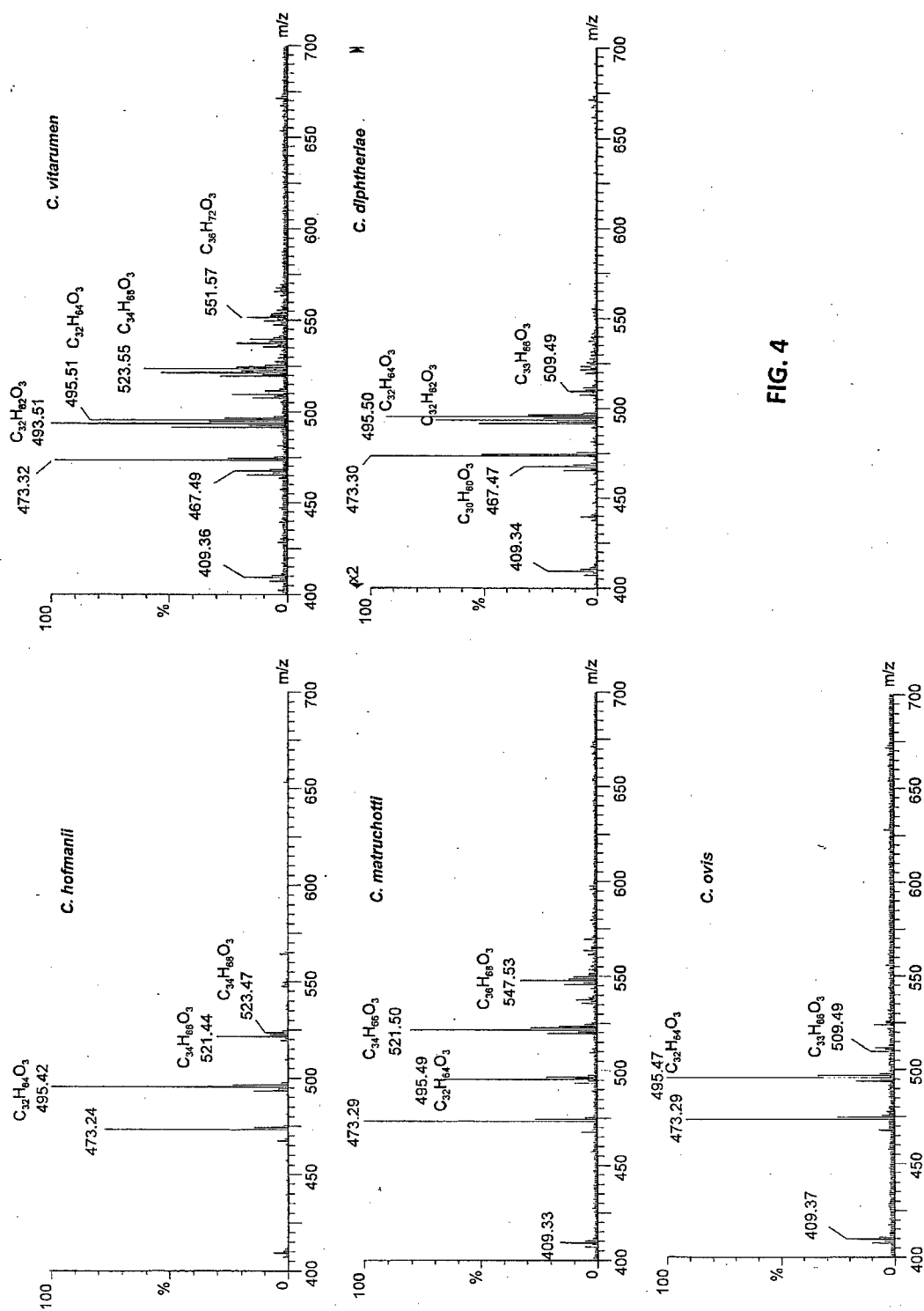


FIG. 4

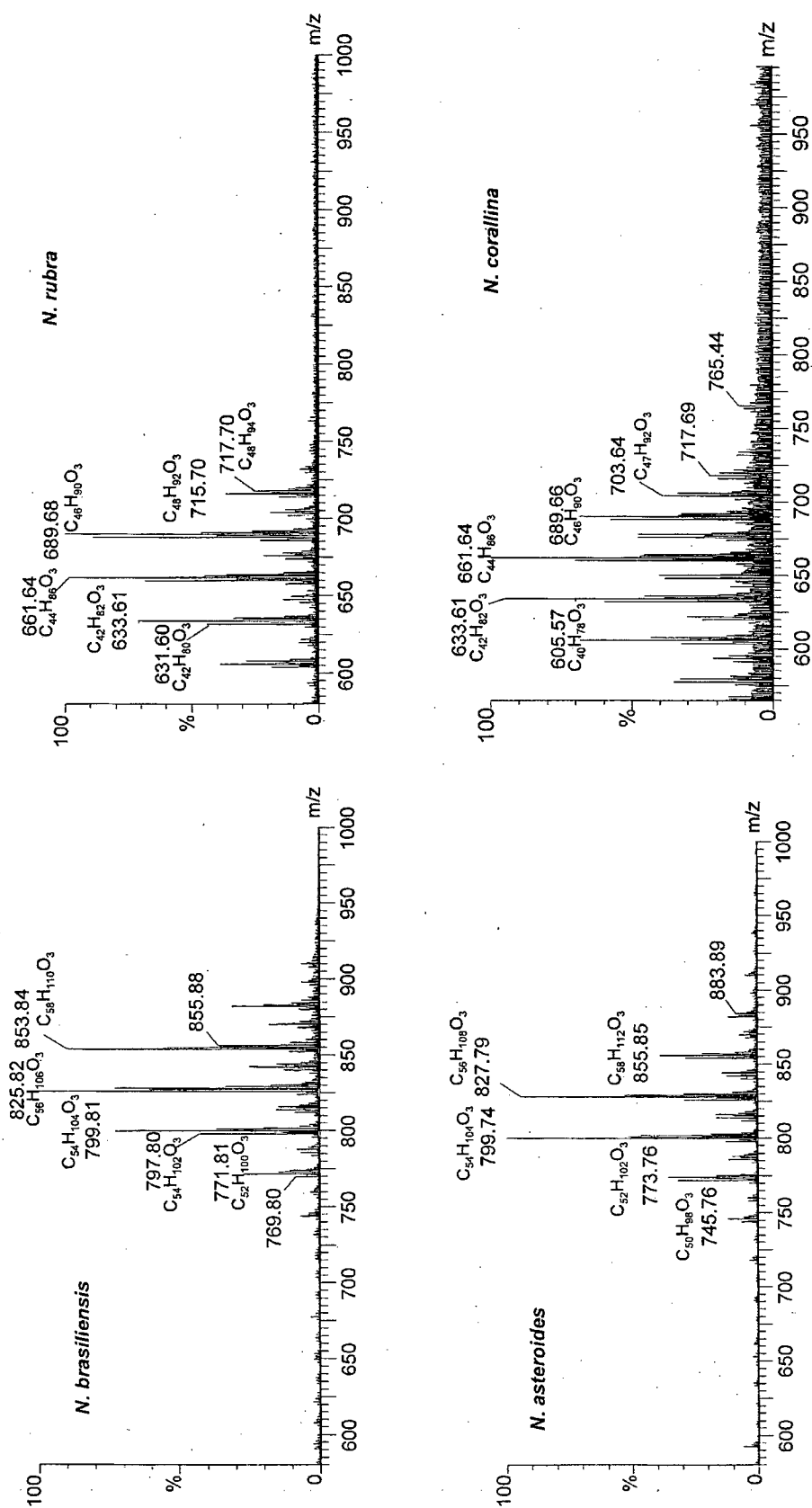


FIG. 5

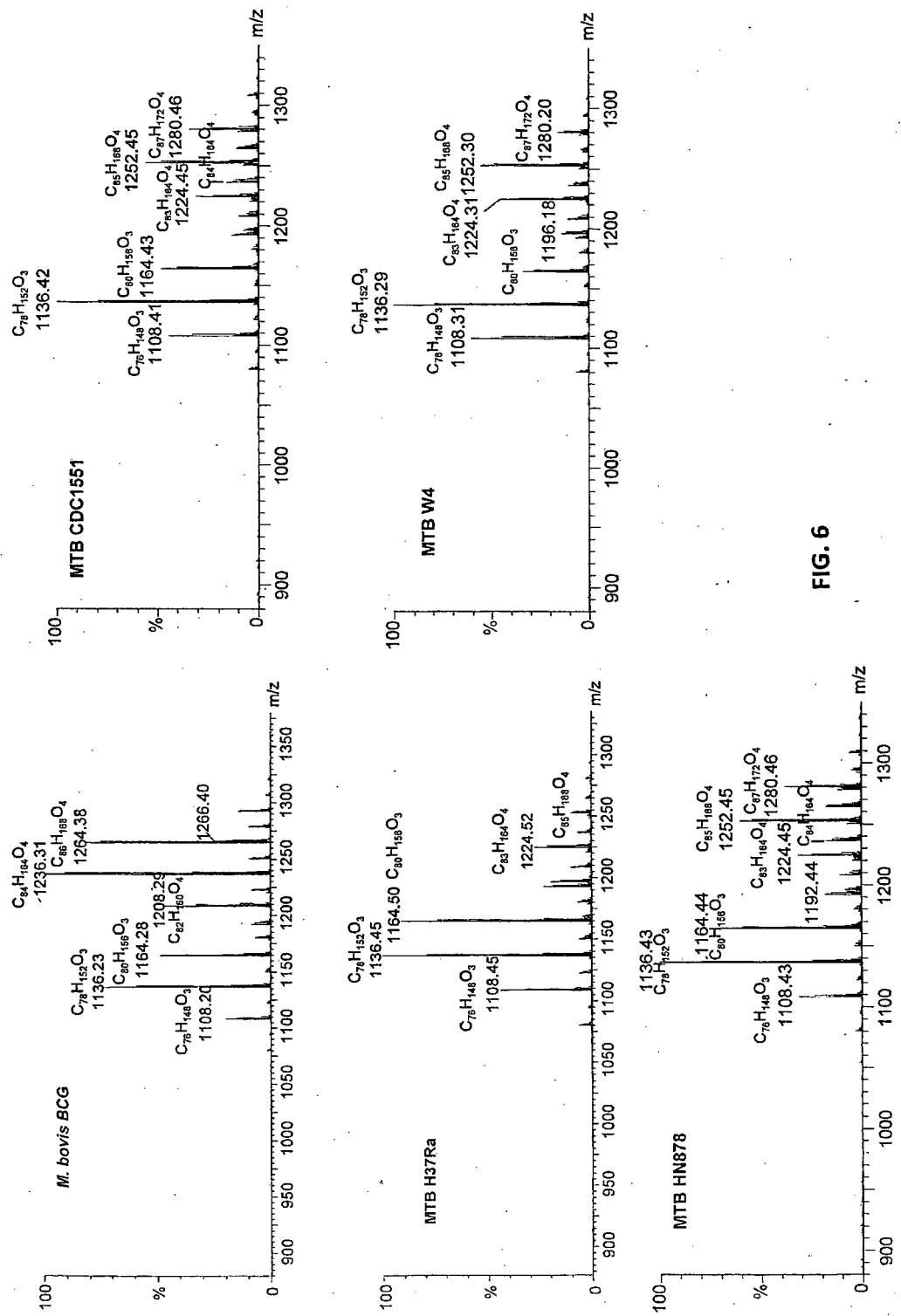


FIG. 6

FIG. 7

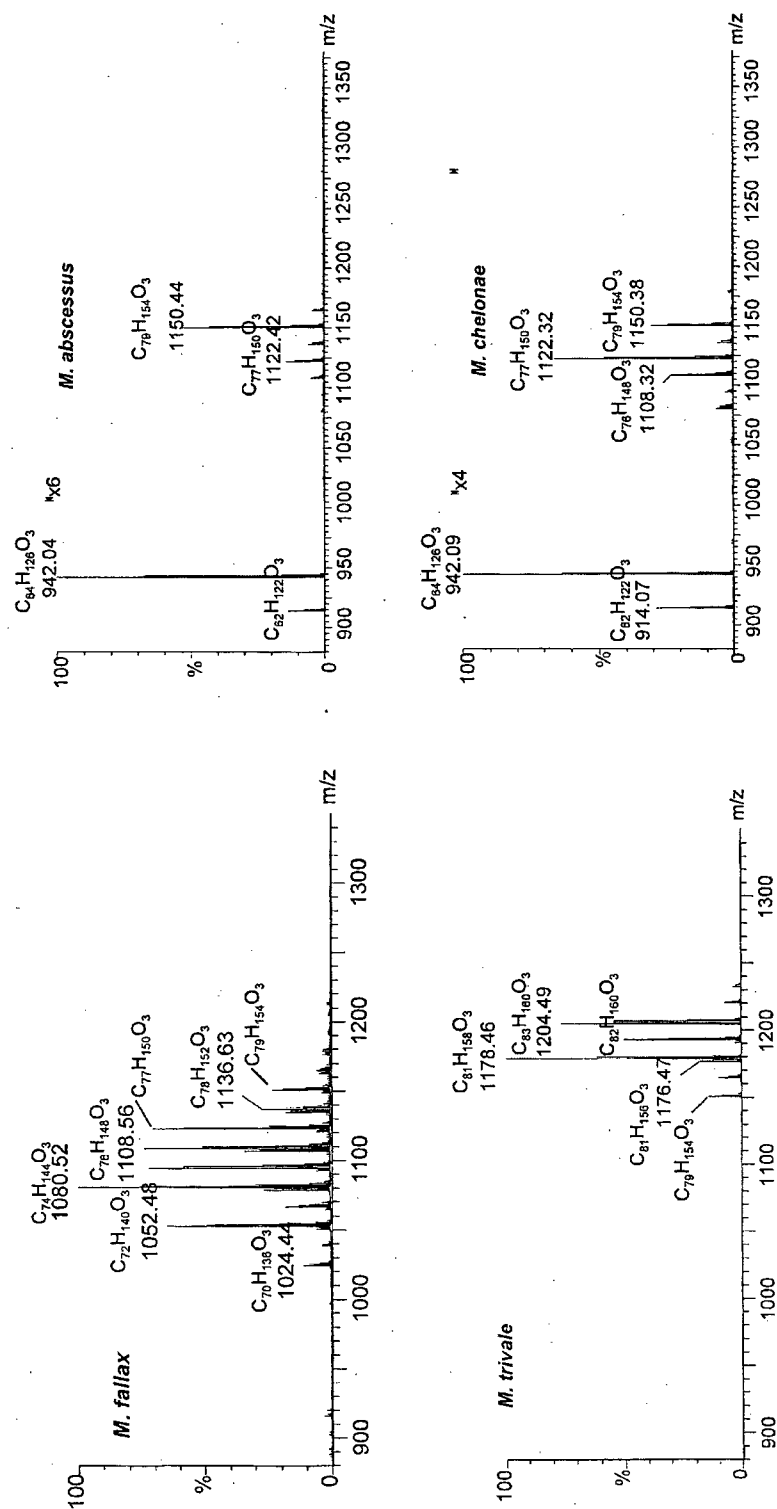
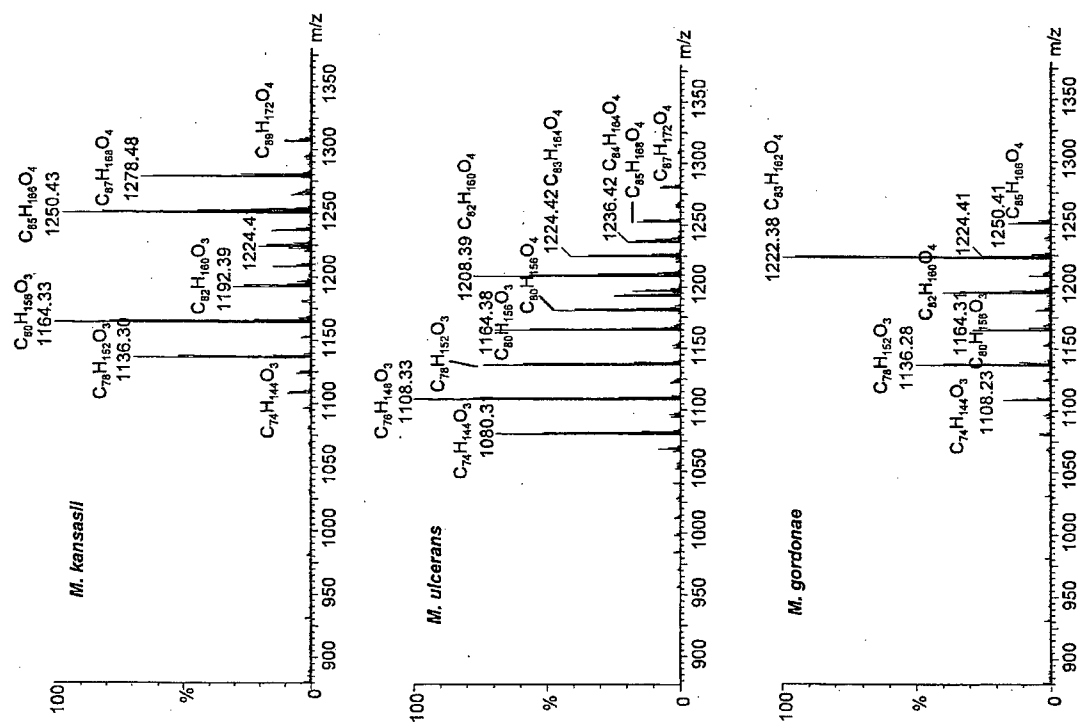


FIG. 8



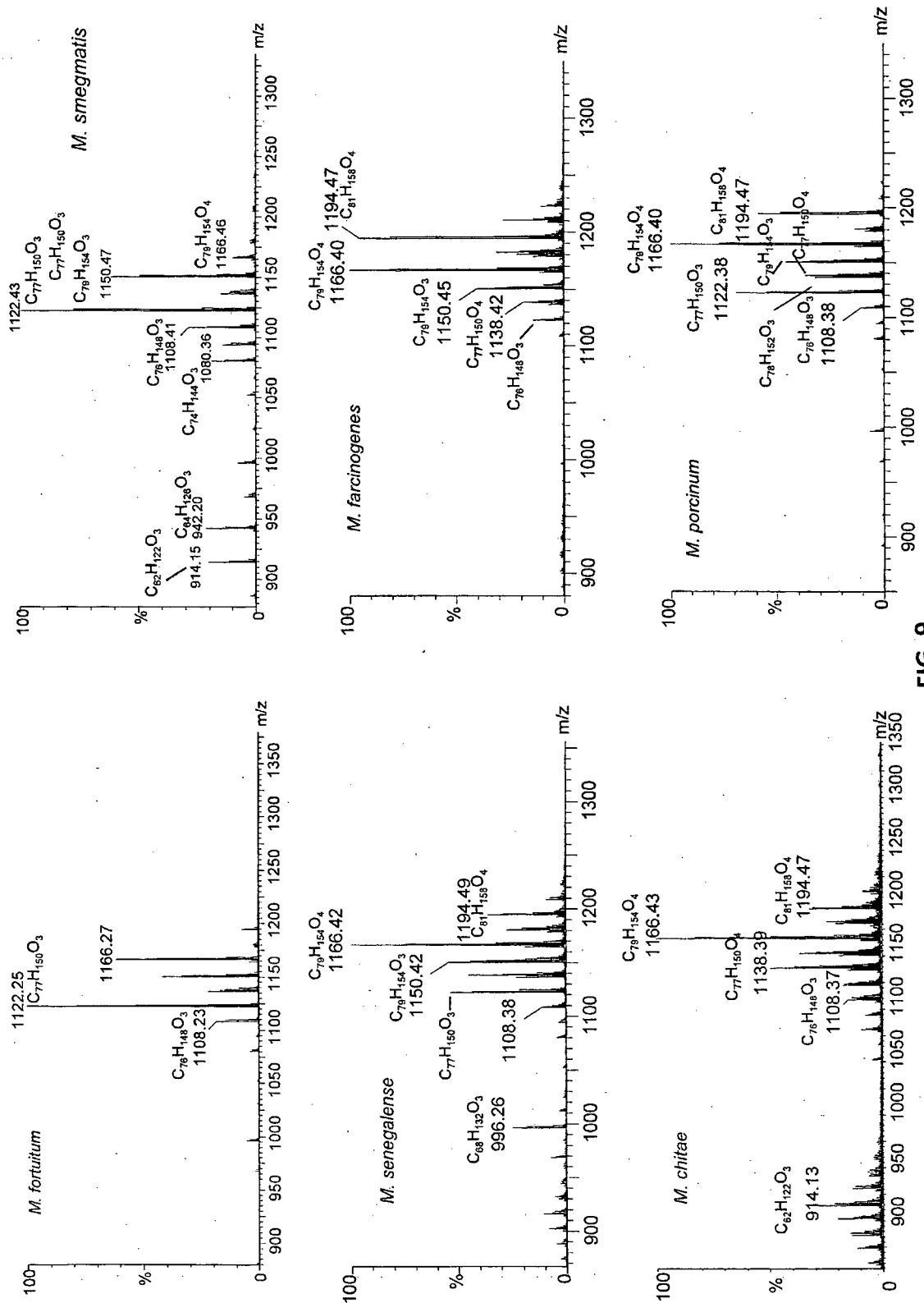


FIG. 9

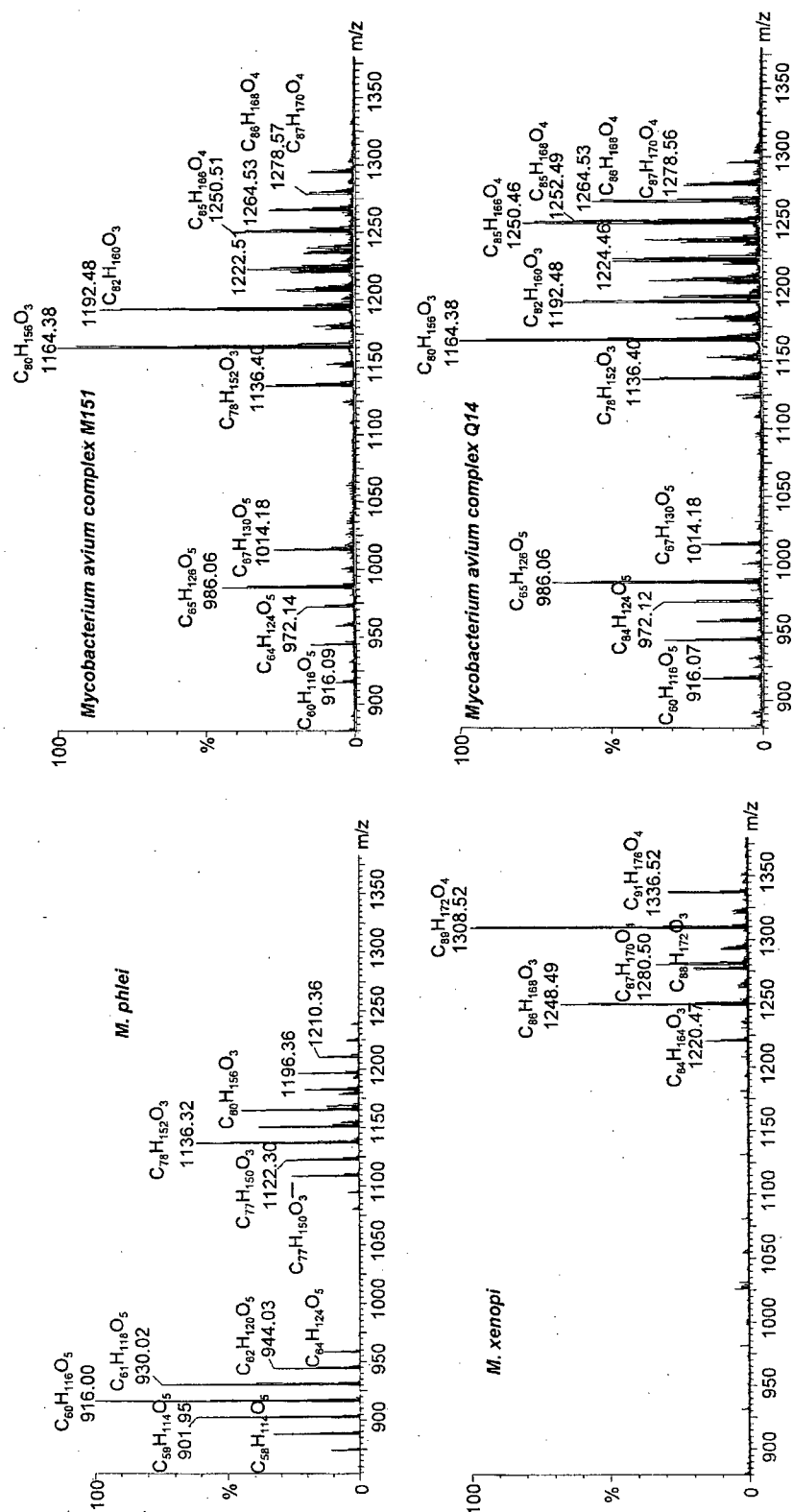


FIG. 10

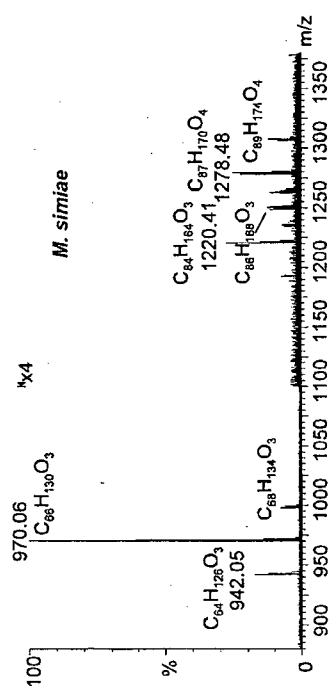


FIG. 11

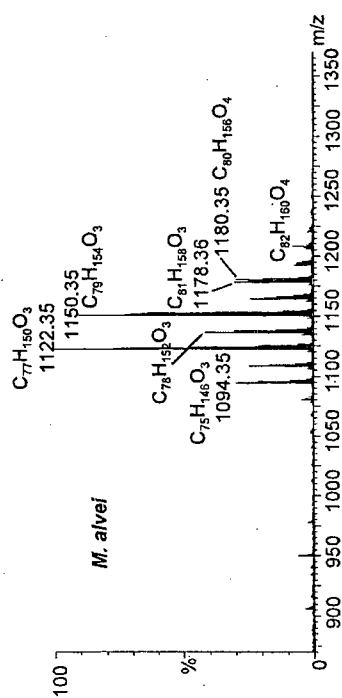


FIG. 12

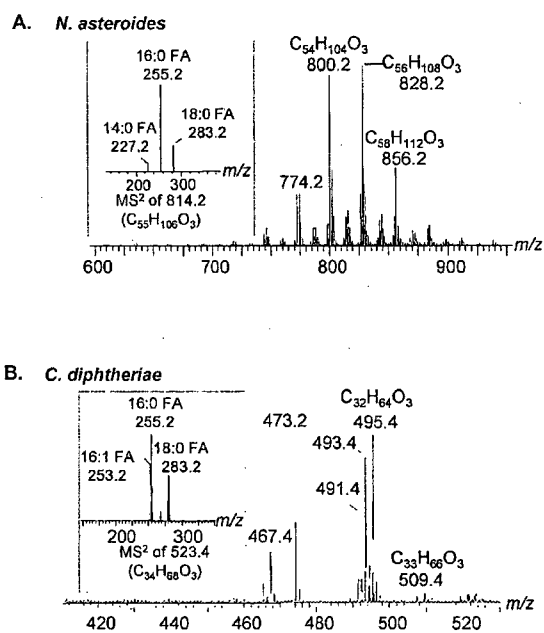


FIG. 13

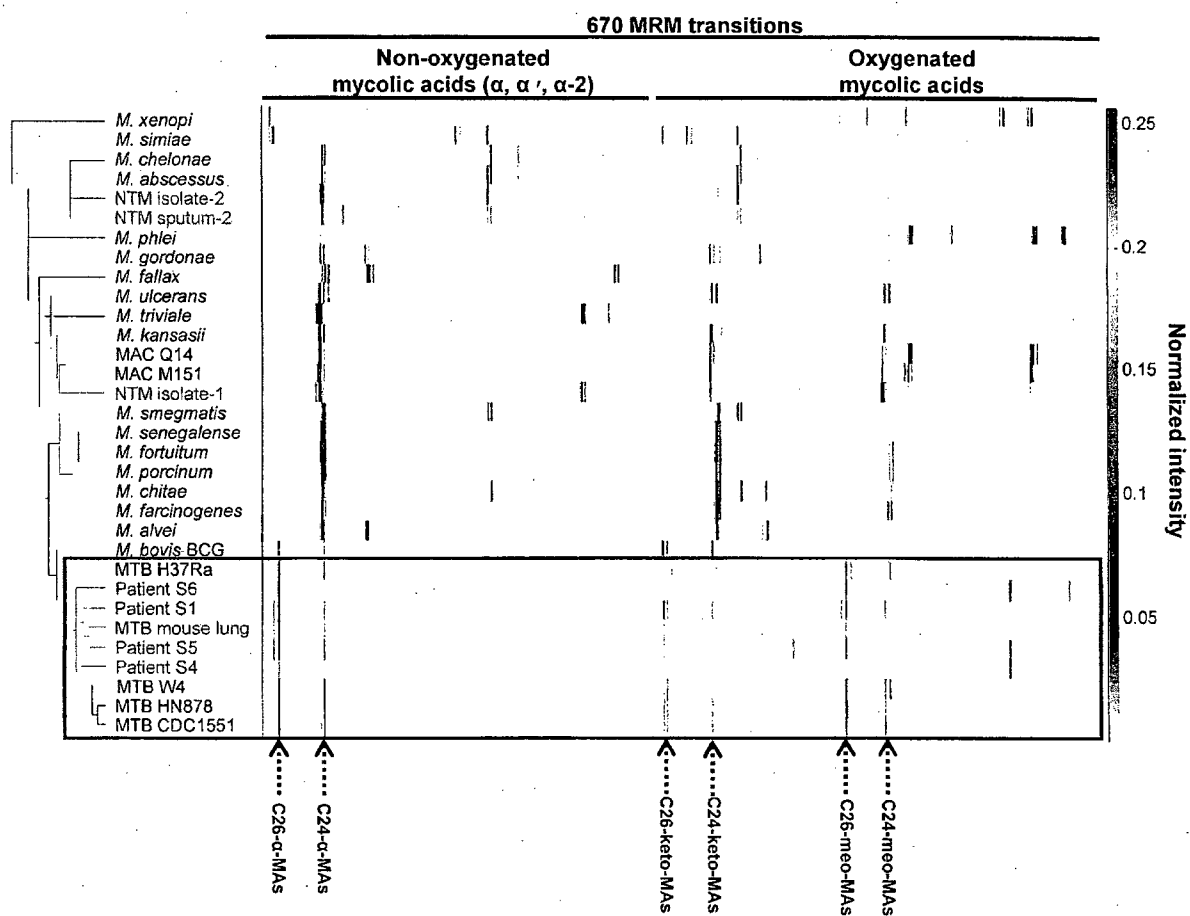


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2012/000034

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. C12Q 1/04 (2006.01) G01N 27/00 (2006.01) ACTION DATE: 21 MARCH 2012 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPODOC, WPI, Medline, Biosis, CAPlus: Mycobacteria, mass spectrometry, mycolic acid and like terms)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Song, SH <i>et al.</i> Electrospray ionization-tandem mass spectrometry analysis of the mycolic acid profiles for the identification of common clinical isolates of mycobacterial species. <i>Journal of Microbiological Methods</i> . 2009 , 77; 165-177 See whole document, particularly Results	1-7 8-21
Y	Walkiewicz, R <i>et al.</i> Application of mycolic acids analysis in diagnostic procedure of tuberculosis and mycobacteriosis – three year experience. <i>Pneumonologia i Alergologia Polska</i> . 2002 , 70 (9-10), 444-449 See Abstract	8-21
P, X	Shui, G <i>et al.</i> Mycolic acids as diagnostic markers for tuberculosis case detection in humans and drug efficacy in mice. <i>EMBO Molecular Medicine</i> . 2011 (December 7) , 4, 27-37	1-21
☒ Further documents are listed in the continuation of Box C ☐ See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 12 April 2012		Date of mailing of the international search report 13/04/2012
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. +61 2 6283 7999		Authorized officer REBECCA HINTON AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6283 2194

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2012/000034

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Shui, G <i>et al.</i> Sensitive profiling of chemically diverse bioactive lipids. Journal of Lipid Research. 2007, 48, 1976-1984	1-21