FIG. 1A

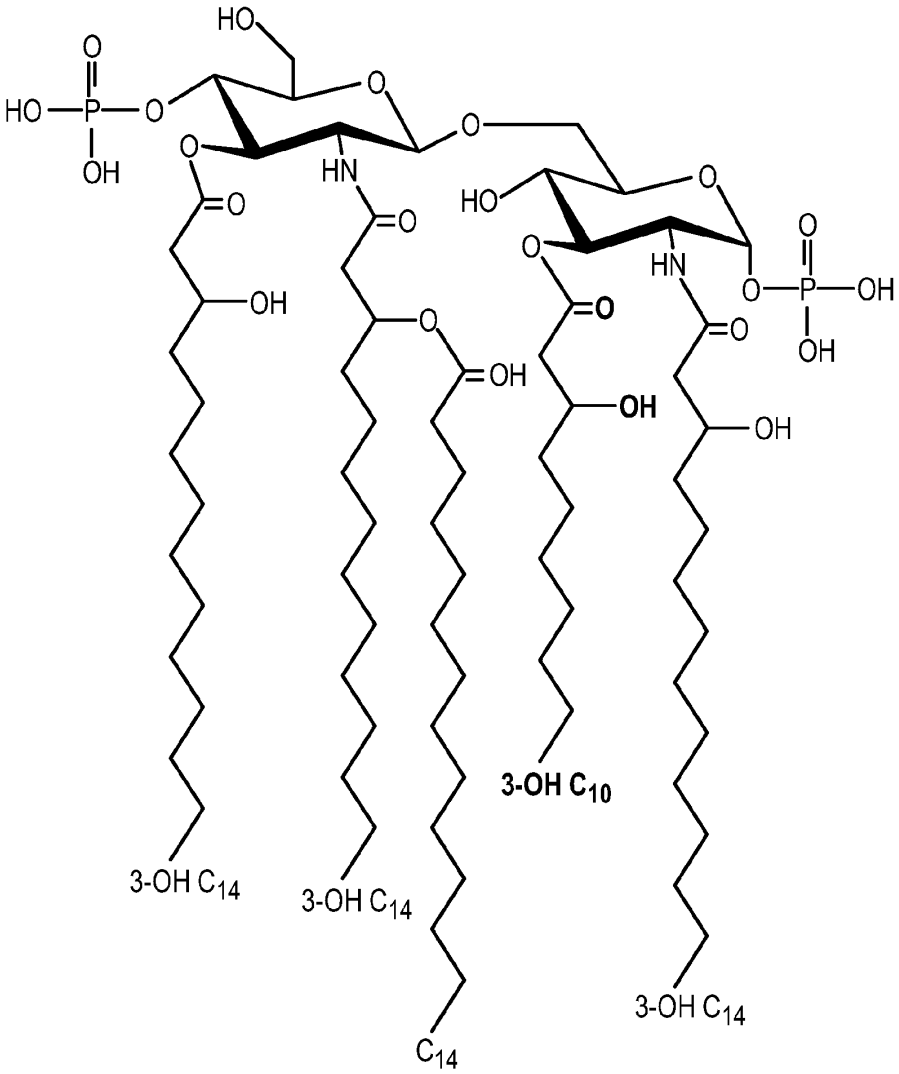
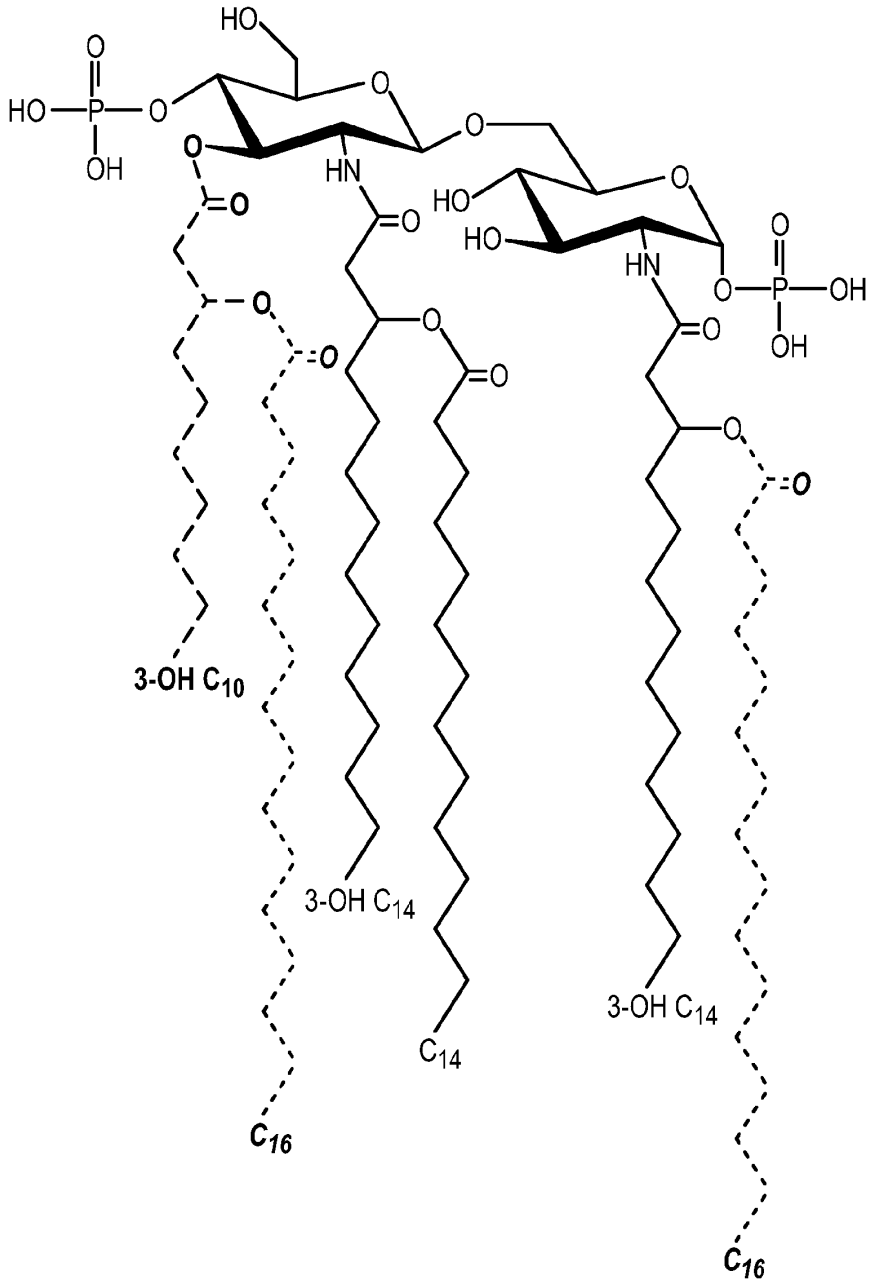

HO
O
HO—P—O
OH
O
HN
O
HO
O
HN
O—P—OH
OH
OH
OH
OH
3-OH C14
3-OH C14
3-OH C10
3-OH C14
C14

FIG. 1B


HO
HO
P
O
OH
O
HN
HO
HO
HN
O
P
OH
OH
3-OH C10
3-OH C14
C14
3-OH C14
C16
C16

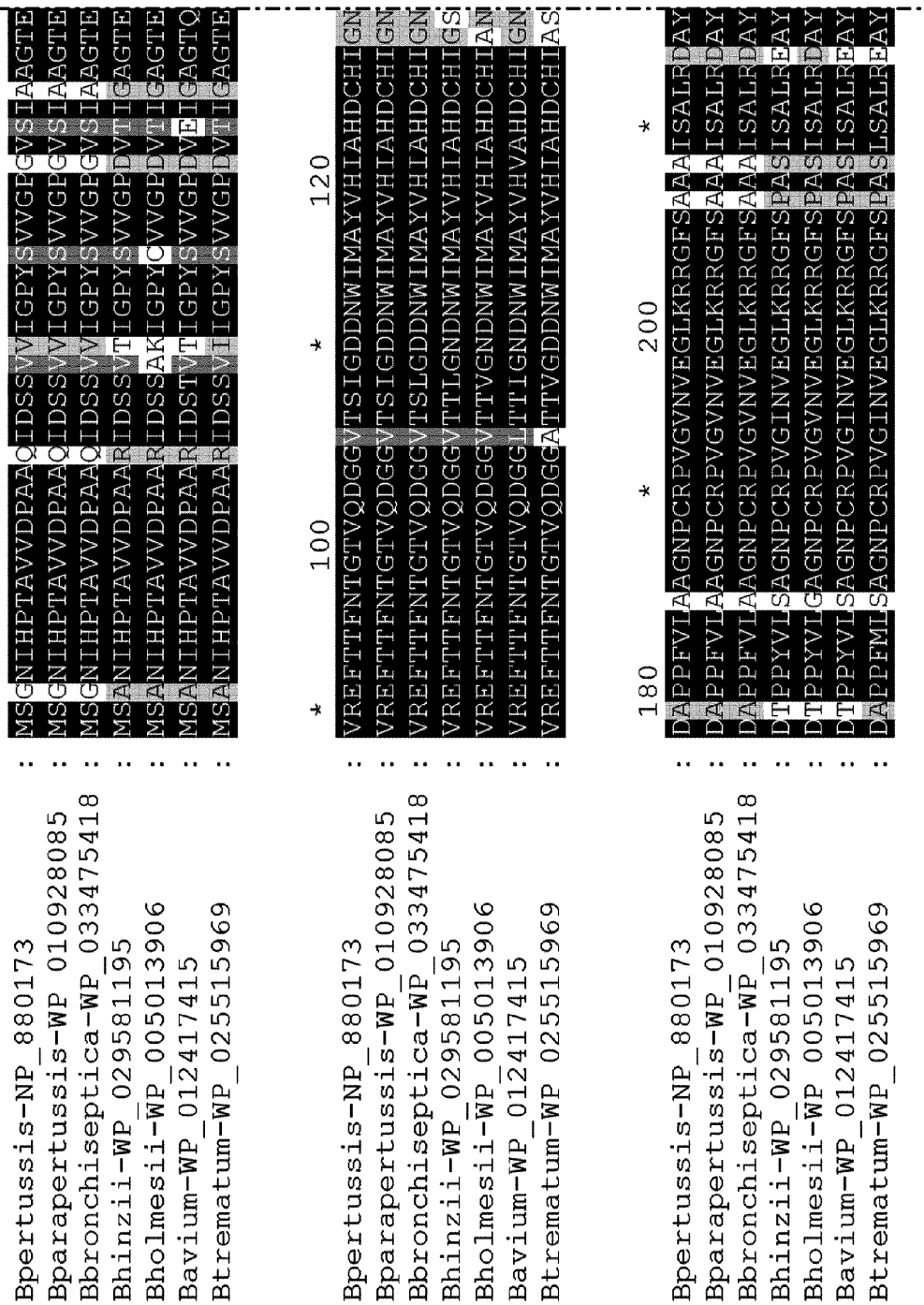

Bpertussis-NP_880173
Bparapertussis-WP_010928085
Bbronchiseptica-WP_033475418
Bhinzii-WP_029581195
Bholmesii-WP_005013906
Bavium-WP_012417415
Btrematum-WP_025515969

FIG. 2

FIG. 2(contd)

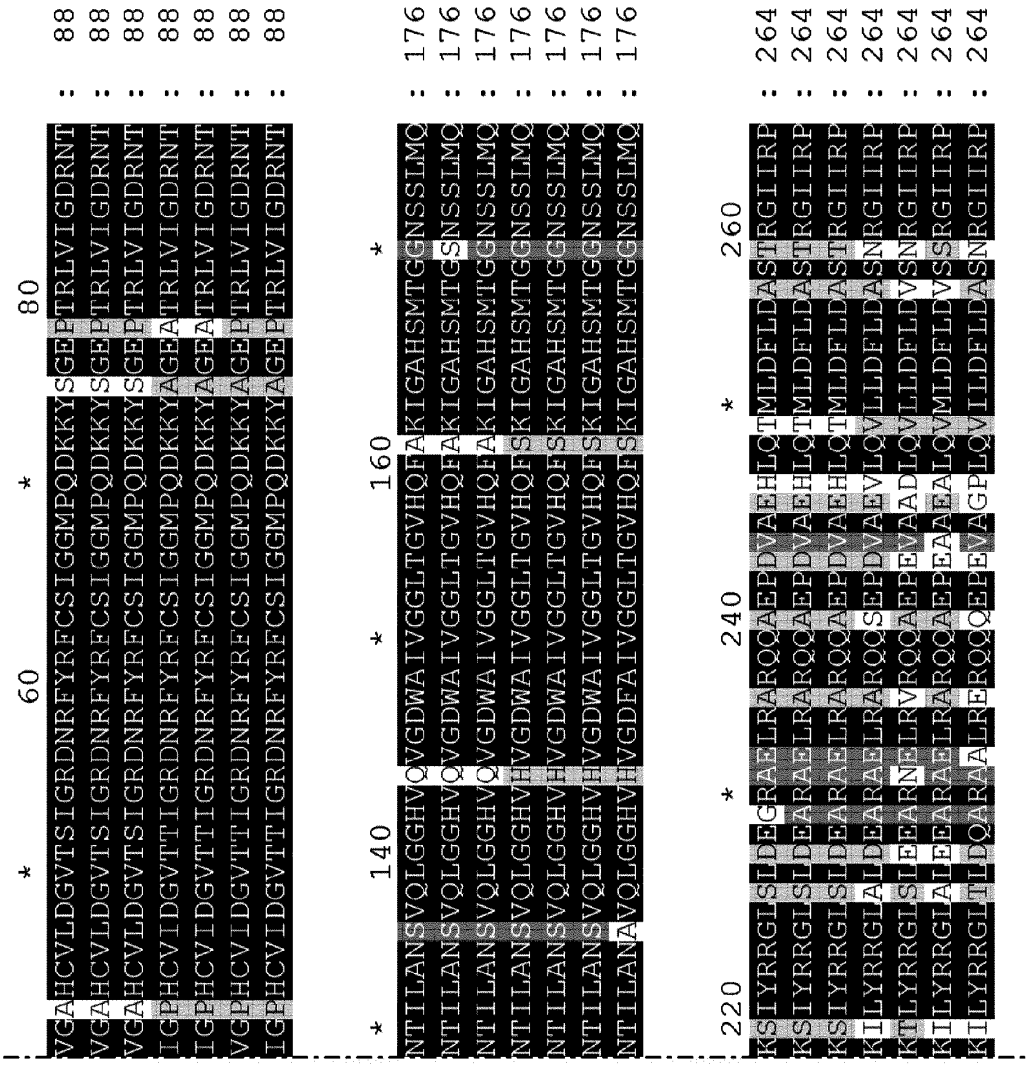


FIG. 3

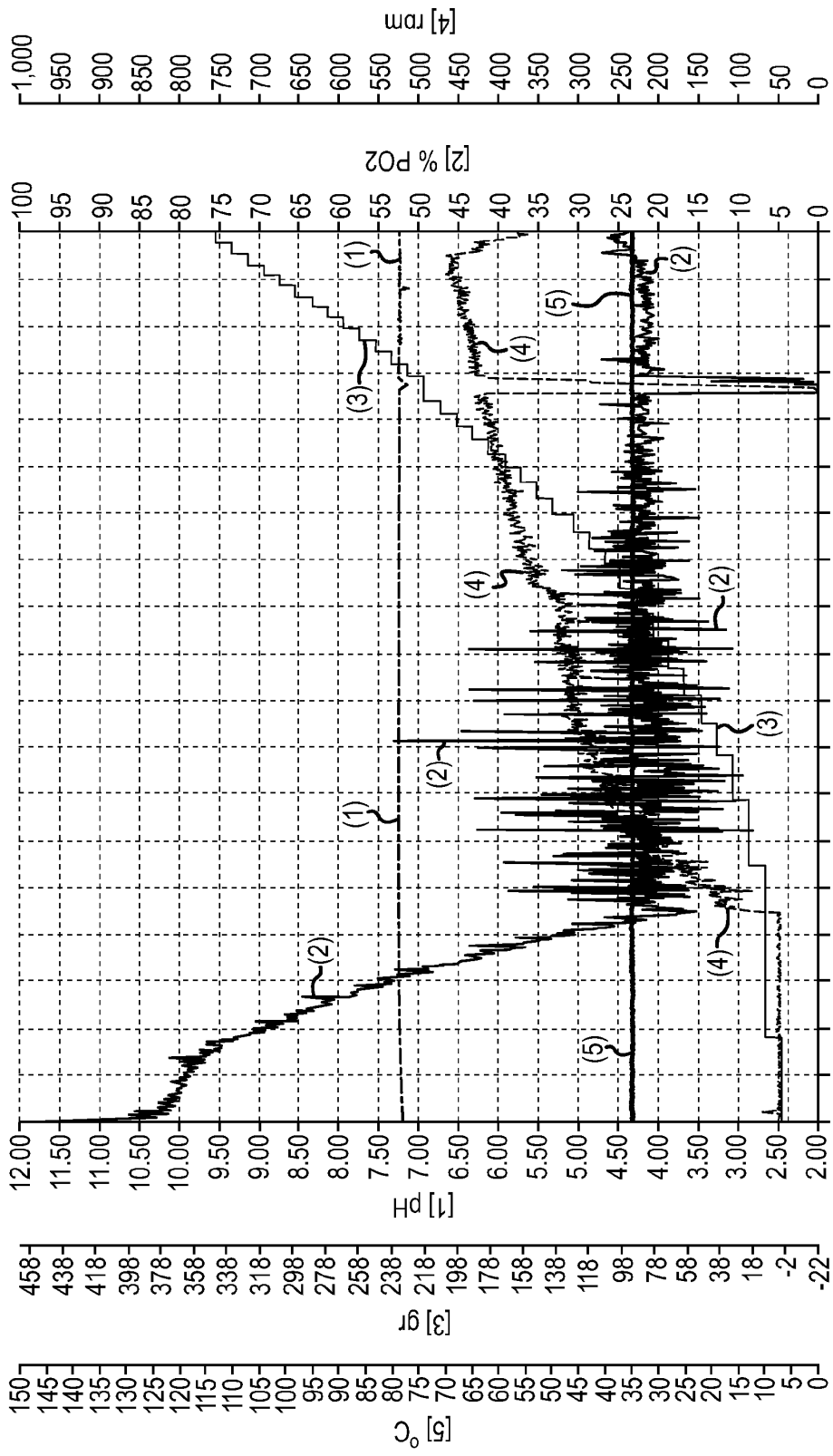


FIG. 3(contd)

KEY			
(1) ---------- [1] pH - Ferm03_AT103\PV: Ferm03 pH measurement		(2) ———— [2]% PO2 - Ferm03_AT104\PV: Ferm03 PO2 measurement	
(3) ———— [3] gr - Ferm03_PdsConsAcid: Weight consumes acid		(4) ------- [4] rpm - Ferm03_ST105\PV: Ferm03 Speed measurement	
(5) ———— [5] °C - Ferm03_TE101\PV: Ferm03 Temperature measurement			
A: 29 Aug. 2018 11:00:00	B: 29 Aug. 2018 13:00:00	C: 29 Aug. 2018 15:00:00	D: 29 Aug. 2018 17:00:00
E: 29 Aug. 2018 19:00:00	F: 29 Aug. 2018 21:00:00	G: 29 Aug. 2018 23:00:00	H: 30 Aug. 2018 01:00:00
I: 30 Aug. 2018 03:00:00	J: 30 Aug. 2018 05:00:00	K: 30 Aug. 2018 07:00:00	L: 30 Aug. 2018 09:00:00
M: 30 Aug. 2018 11:00:00	N: 30 Aug. 2018 13:00:00	N: 30 Aug. 2018 15:00:00	P: 30 Aug. 2018 17:00:00
Q: 30 Aug. 2018 19:00:00	R: 30 Aug. 2018 21:00:00	S: 30 Aug. 2018 23:00:00	

FIG. 4

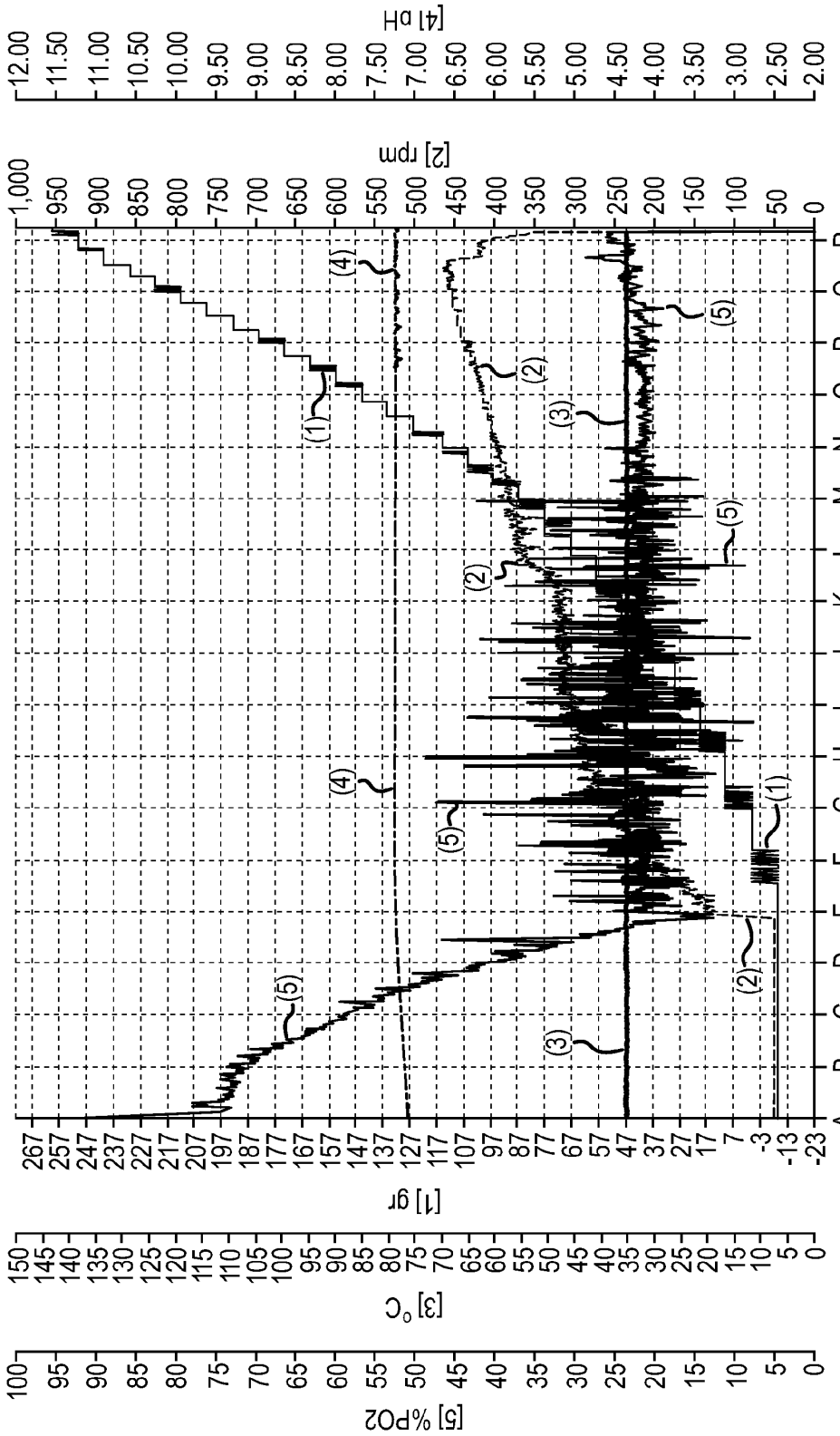


FIG. 4(contd)

KEY			
(1) ——— [1] gr - Ferm02_PdsConsAcid: Weight consumes acid		(2) ------- [2] rpm - Ferm02_ST105\PV: Ferm02 Speed measurement	
(3) ——— [3] °C - Ferm02_TE101\PV: Ferm02 Temperature measurement		(4) -·-·-·- [4] pH - Ferm02_AT103\PV: Ferm02 pH measurement	
(5) ——— [5]% PO2 - Ferm02_AT104\PV: Ferm02 PO2 measurement			
A: 07 Nov. 2018 10:46:57	B: 07 Nov. 2018 12:46:57	C: 07 Nov. 2018 14:46:57	D: 07 Nov. 2018 16:46:57
E: 07 Nov. 2018 18:46:57	F: 07 Nov. 2018 20:46:57	G: 07 Nov. 2018 22:46:57	H: 08 Nov. 2018 00:46:57
I: 08 Nov. 2018 02:46:57	J: 08 Nov. 2018 04:46:57	K: 08 Nov. 2018 06:46:57	L: 08 Nov. 2018 08:46:57
M: 07 Nov. 2018 10:46:57	N: 07 Nov. 2018 12:46:57	N: 07 Nov. 2018 14:46:57	P: 08 Nov. 2018 16:46:57
Q: 08 Nov. 2018 18:46:57	R: 08 Nov. 2018 20:46:57		

FIG. 5

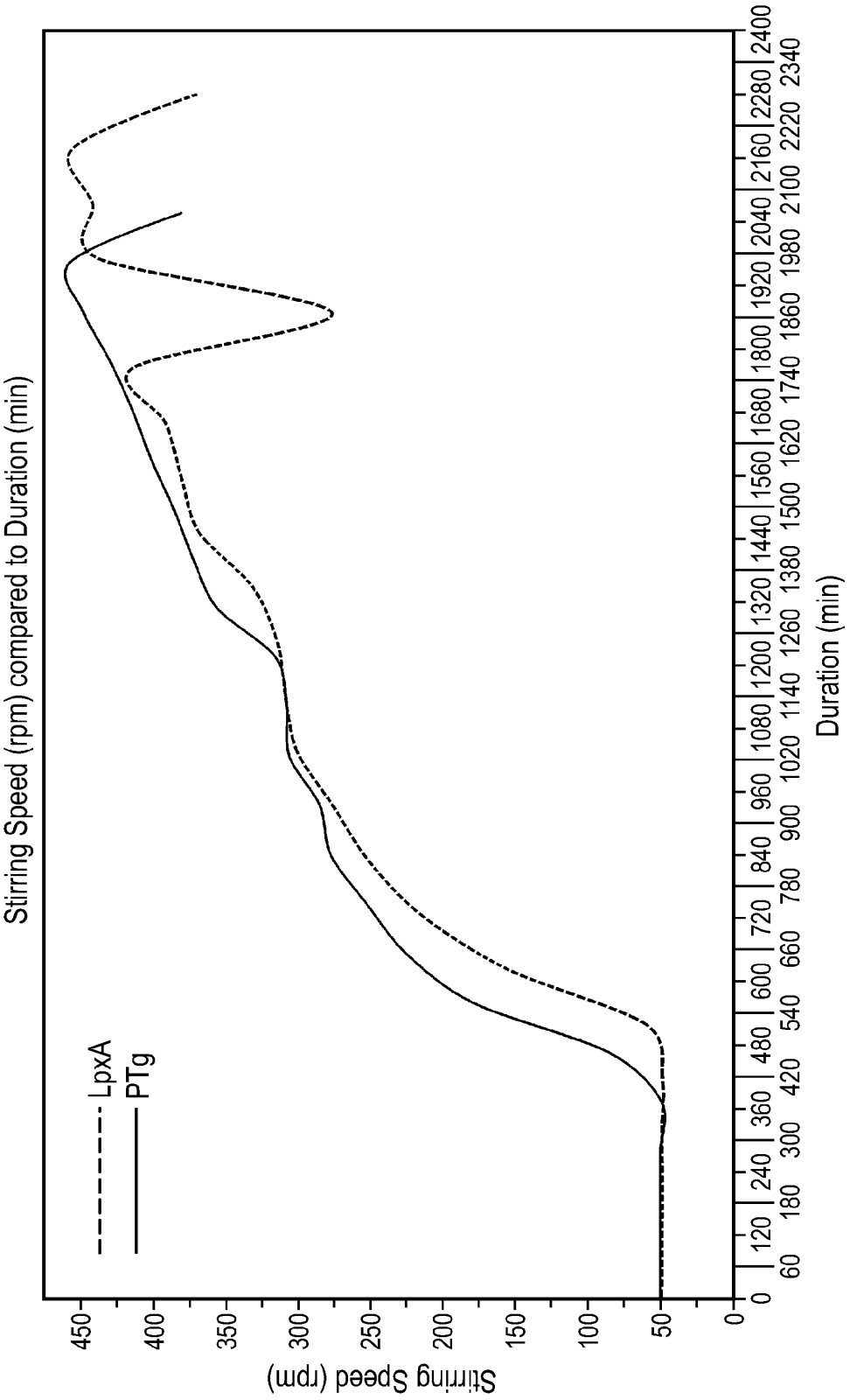
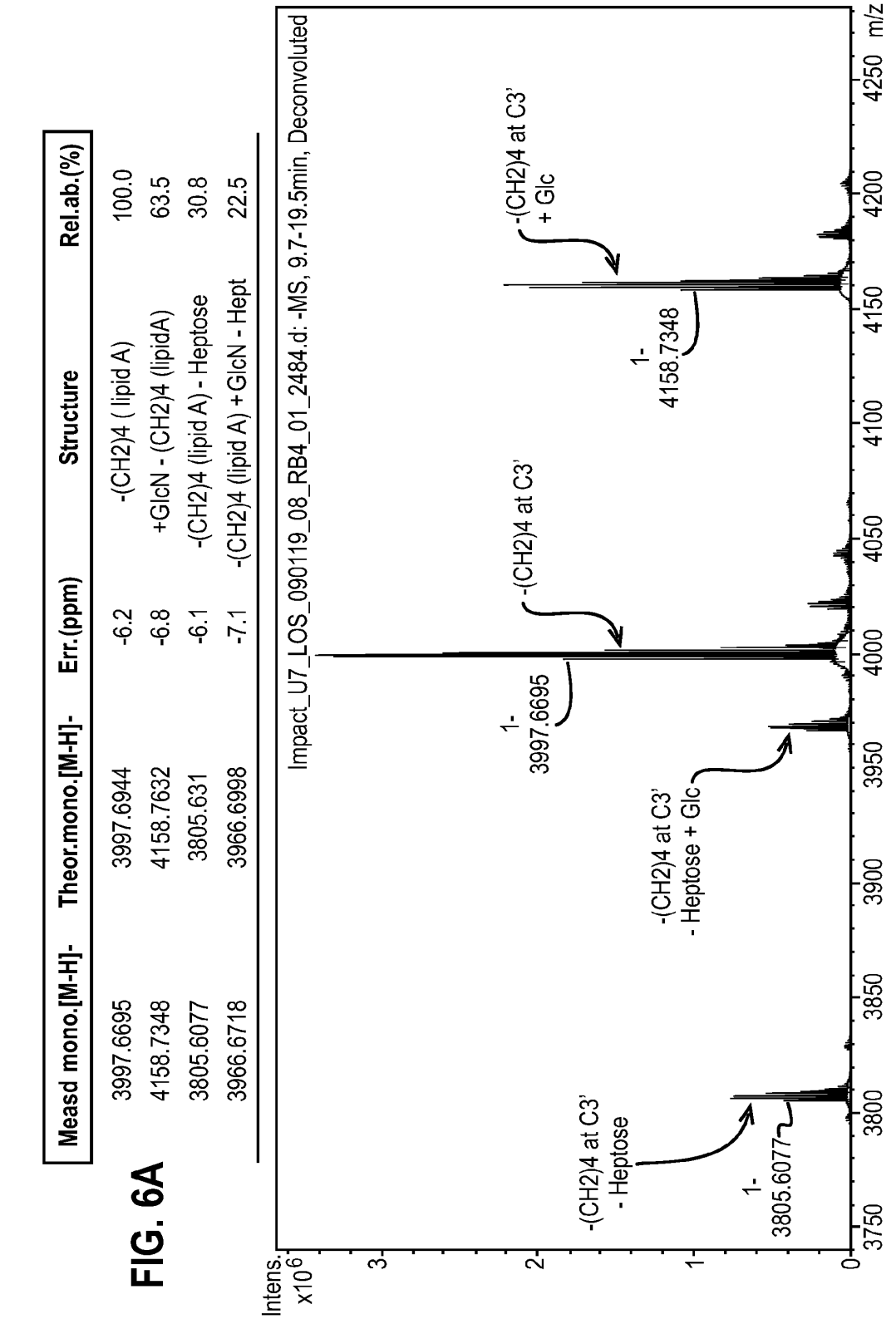

Stirring Speed (rpm) compared to Duration (min)
LpxA
PTg
Stirring Speed (rpm)
0
50
100
150
200
250
300
350
400
450
Duration (min)
0
60
120
180
240
300
360
420
480
540
600
660
720
780
840
900
960
1020
1080
1140
1200
1260
1320
1380
1440
1500
1560
1620
1680
1740
1800
1860
1920
1980
2040
2100
2160
2220
2280
2340
2400

FIG. 6A

Measd mono.[M-H]-	Theor.mono.[M-H]-	Err.(ppm)	Structure	Rel.ab.(%)
3997.6695	3997.6944	-6.2	-(CH2)4 (lipid A)	100.0
4158.7348	4158.7632	-6.8	+GlcN - (CH2)4 (lipidA)	63.5
3805.6077	3805.631	-6.1	-(CH2)4 (lipid A) - Heptose	30.8
3966.6718	3966.6998	-7.1	-(CH2)4 (lipid A) +GlcN - Hept	22.5



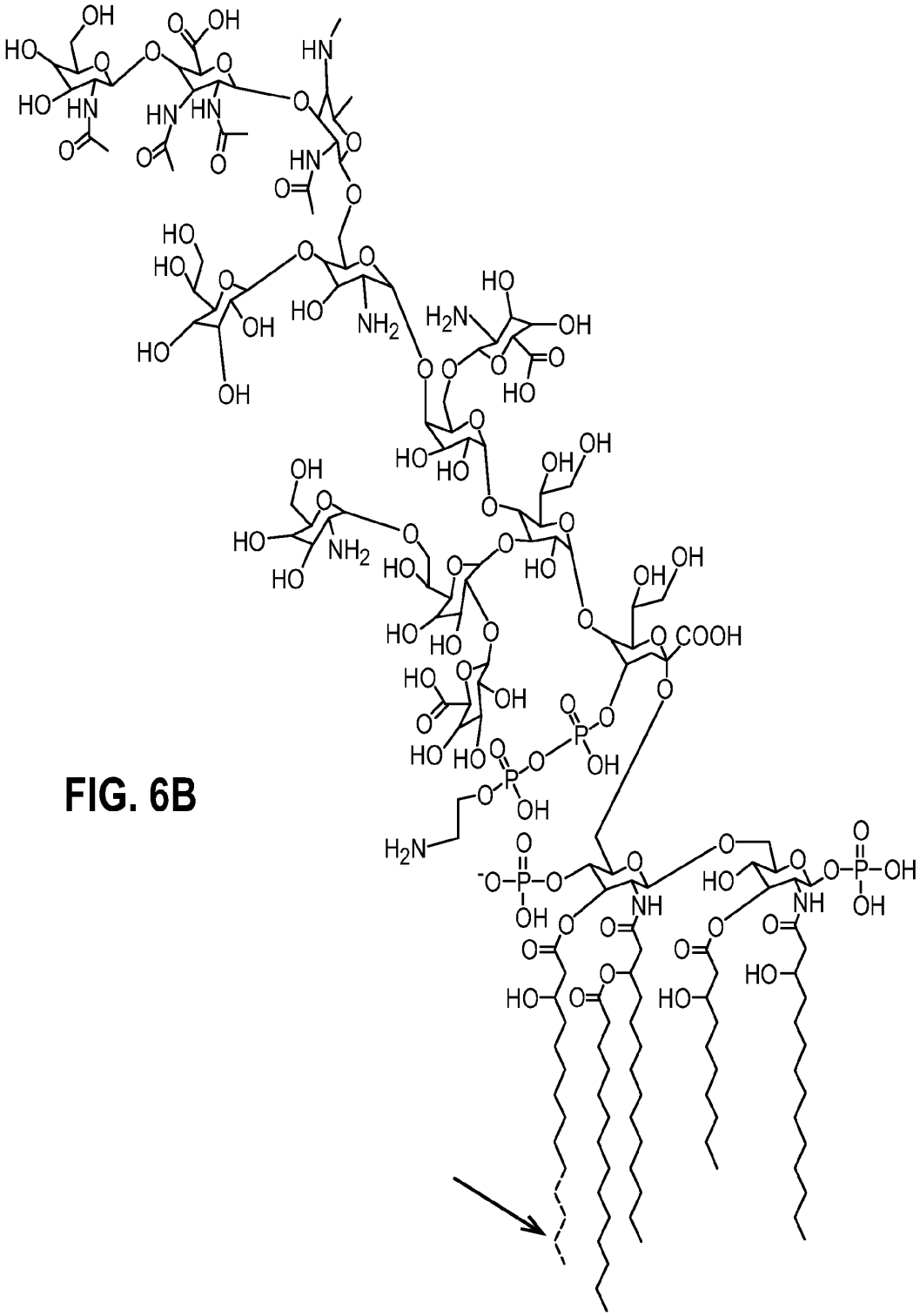
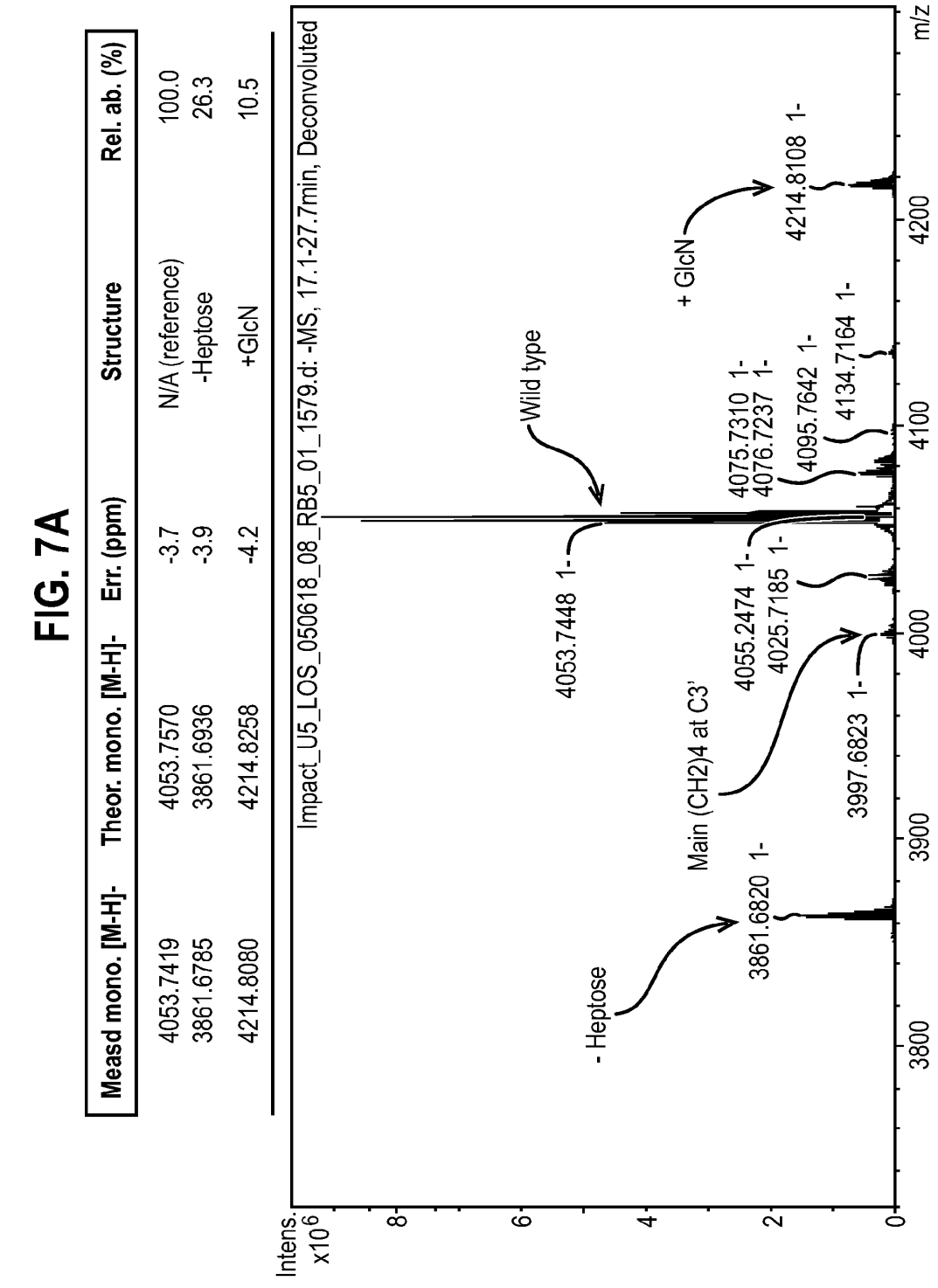


FIG. 6B

Exact Mass: 4053.7570

FIG. 7A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
4053.7419	4053.7570	-3.7	N/A (reference)	100.0
3861.6785	3861.6936	-3.9	-Heptose	26.3
4214.8080	4214.8258	-4.2	+GlcN	10.5



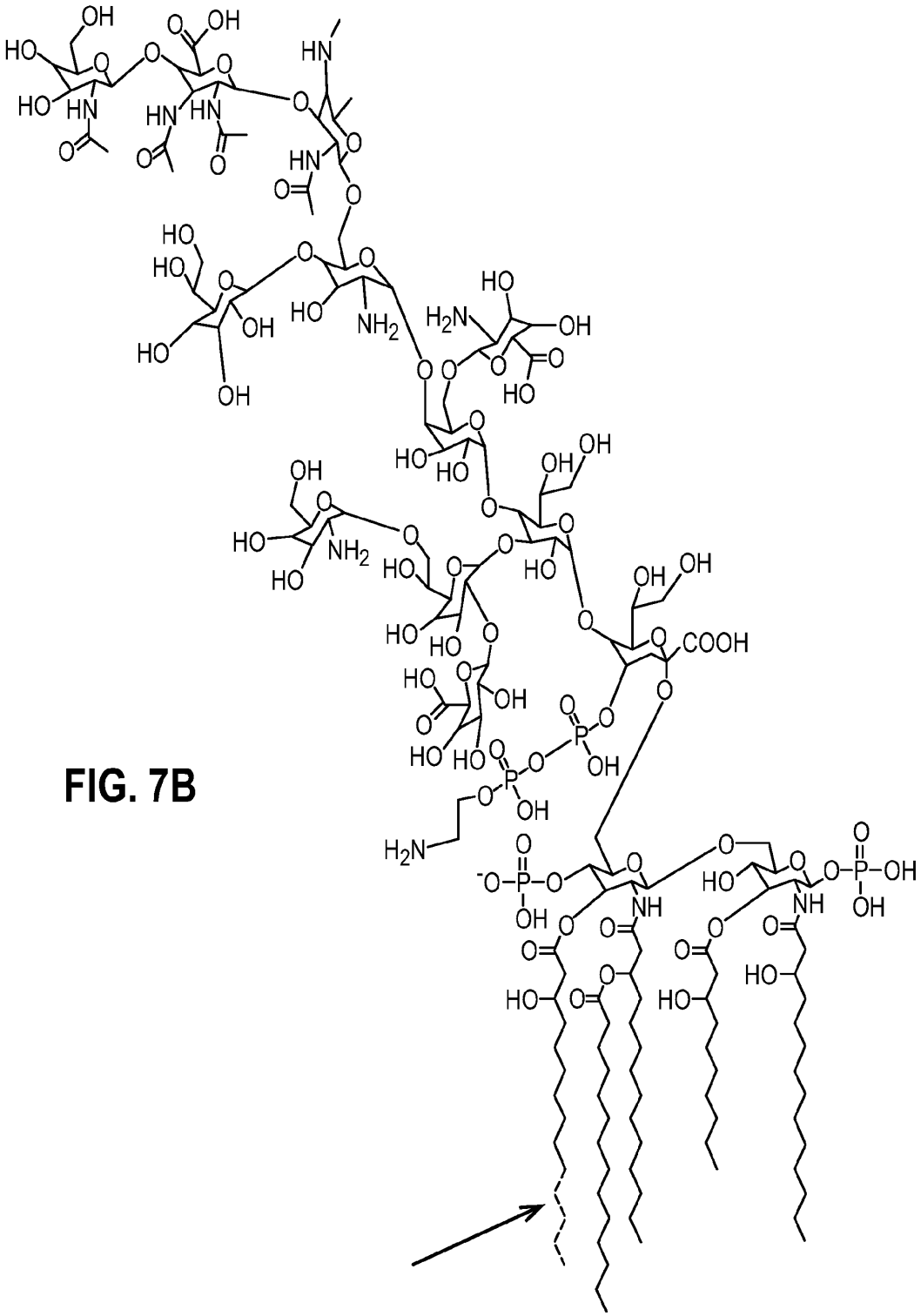
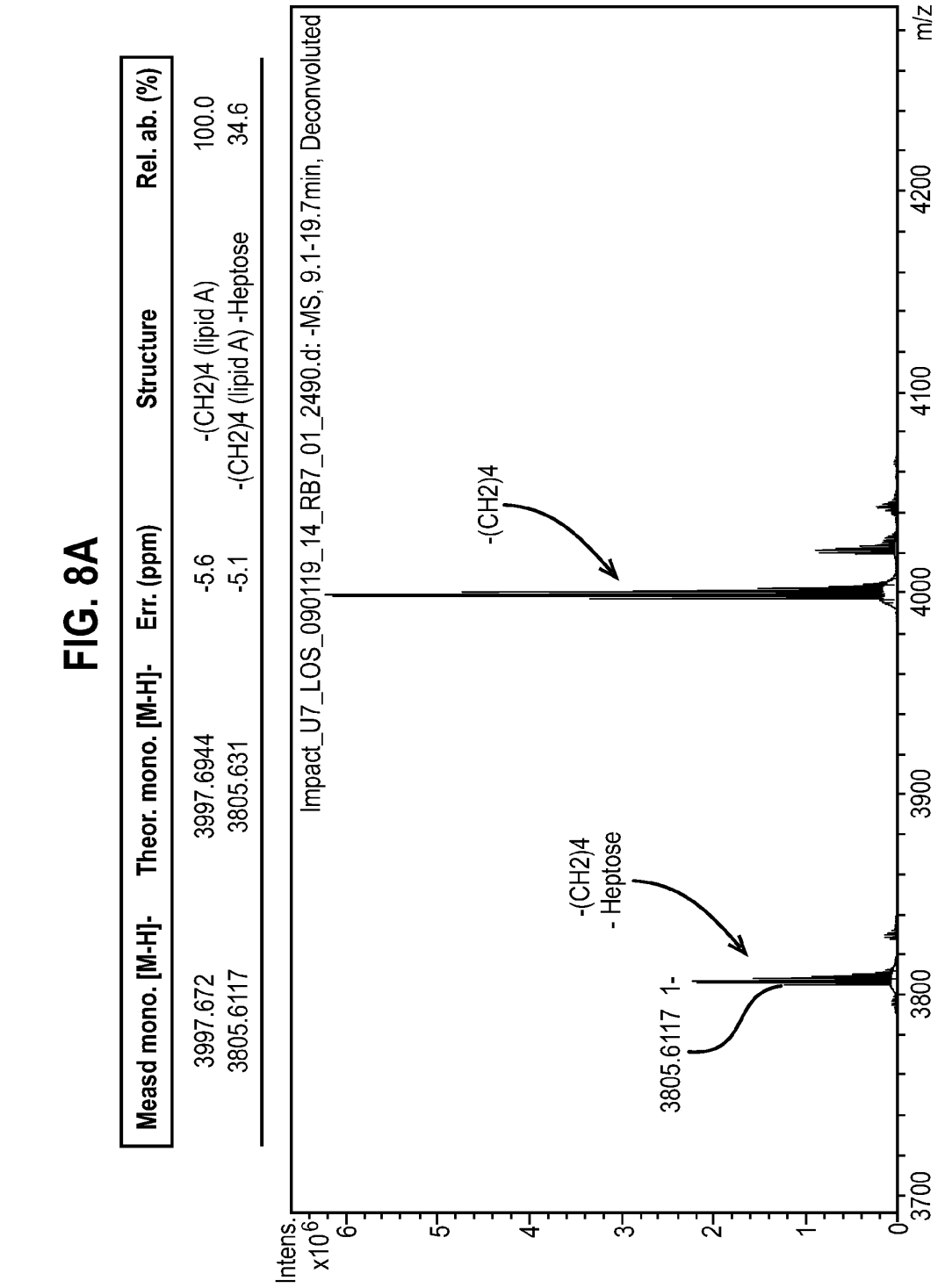


FIG. 7B

Exact Mass: 4053.7570

FIG. 8A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
3997.672	3997.6944	-5.6	-(CH2)4 (lipid A)	100.0
3805.6117	3805.631	-5.1	-(CH2)4 (lipid A) -Heptose	34.6



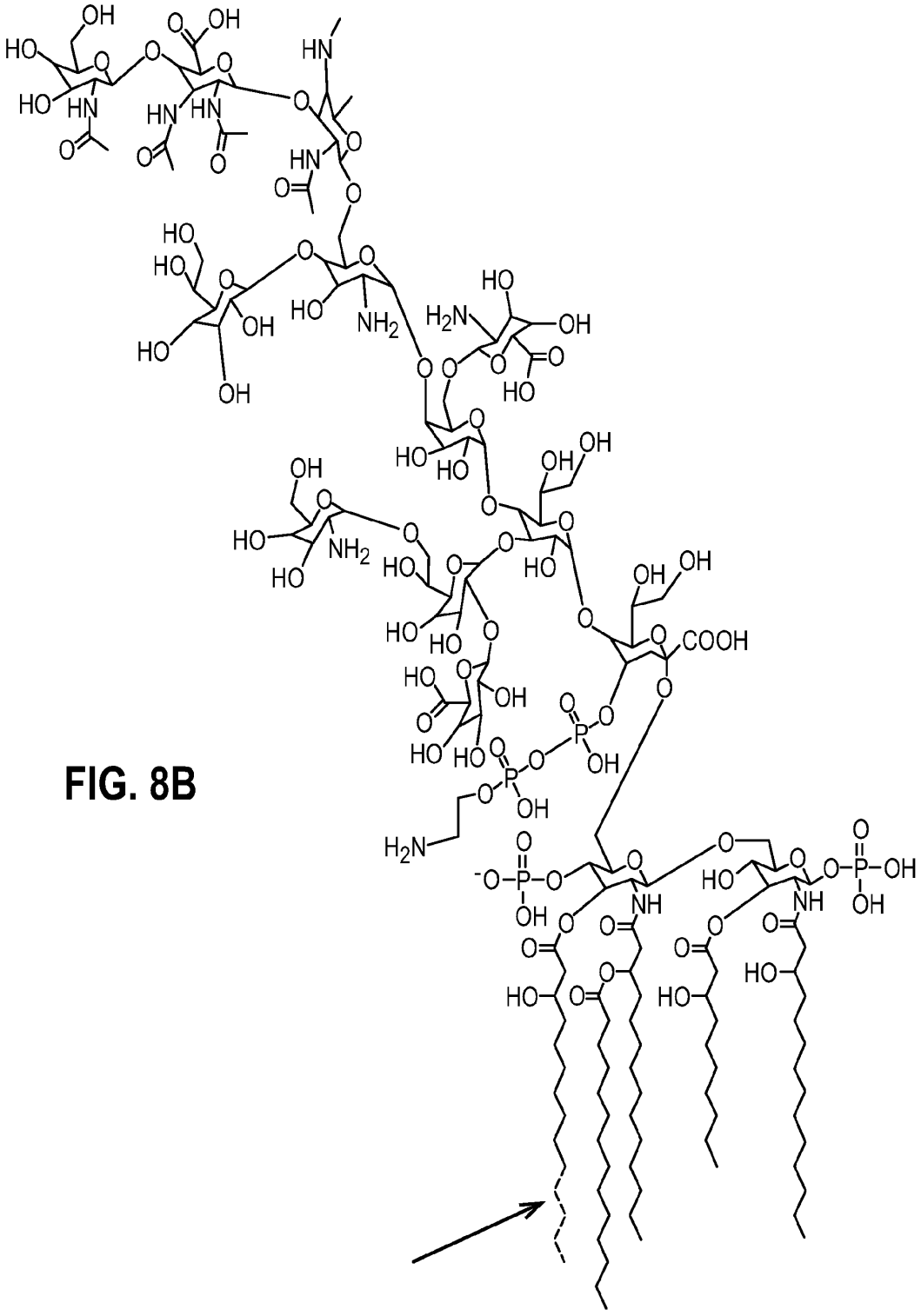
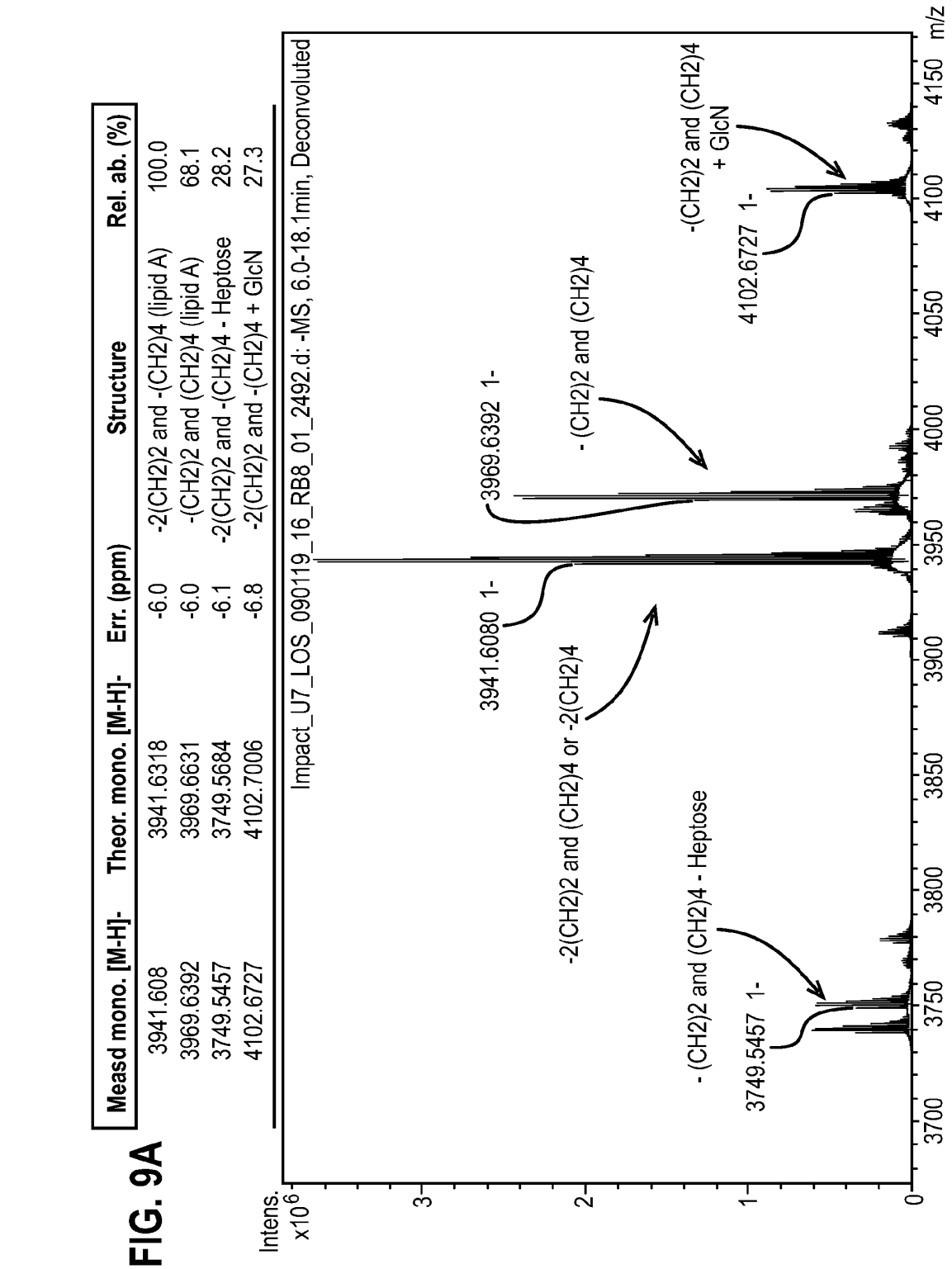


FIG. 8B

Exact Mass: 4053.7570

FIG. 9A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
3941.608	3941.6318	-6.0	-2(CH2)2 and -(CH2)4 (lipid A)	100.0
3969.6392	3969.6631	-6.0	-(CH2)2 and (CH2)4 (lipid A)	68.1
3749.5457	3749.5684	-6.1	-2(CH2)2 and -(CH2)4 - Heptose	28.2
4102.6727	4102.7006	-6.8	-2(CH2)2 and -(CH2)4 + GlcN	27.3



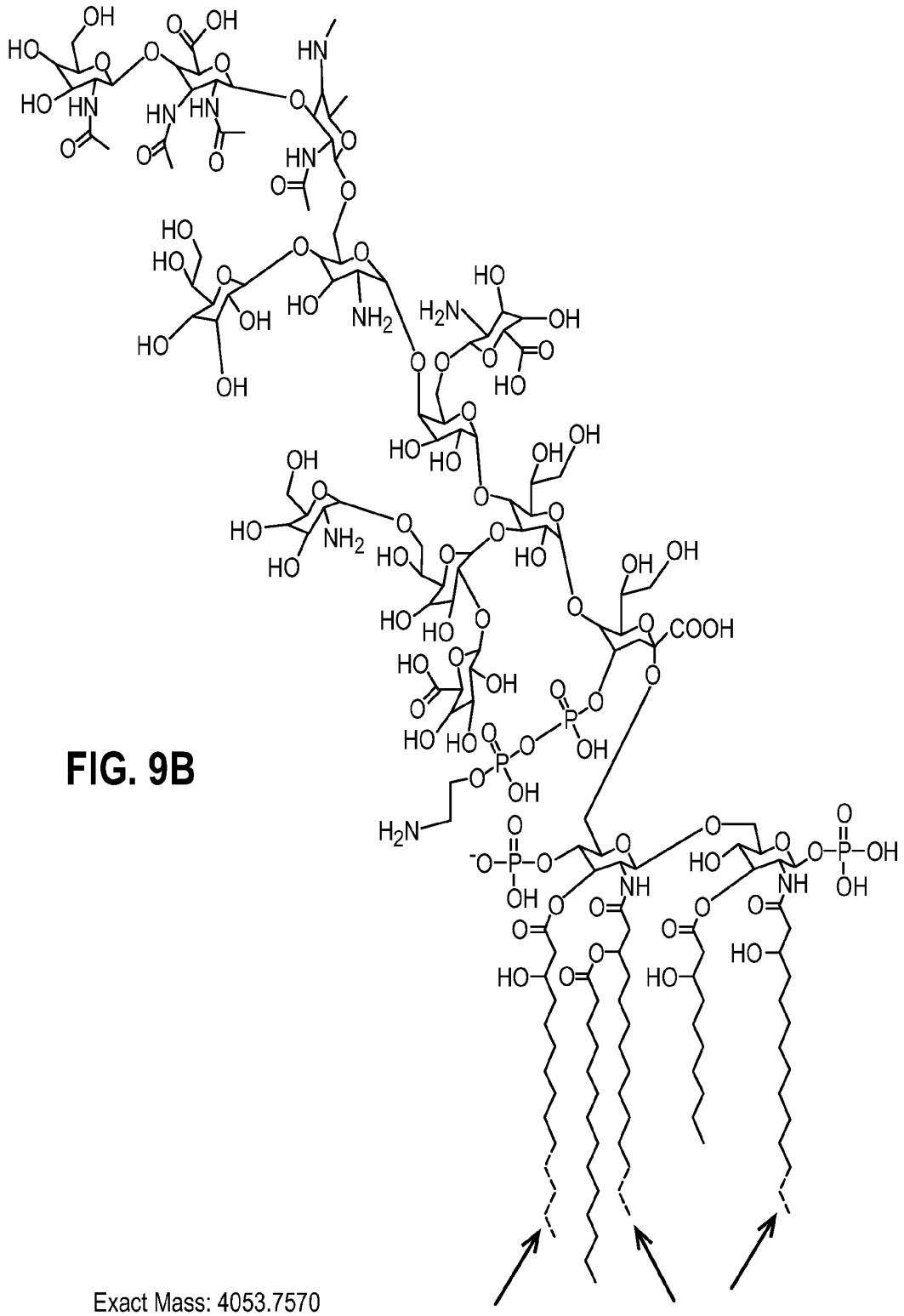
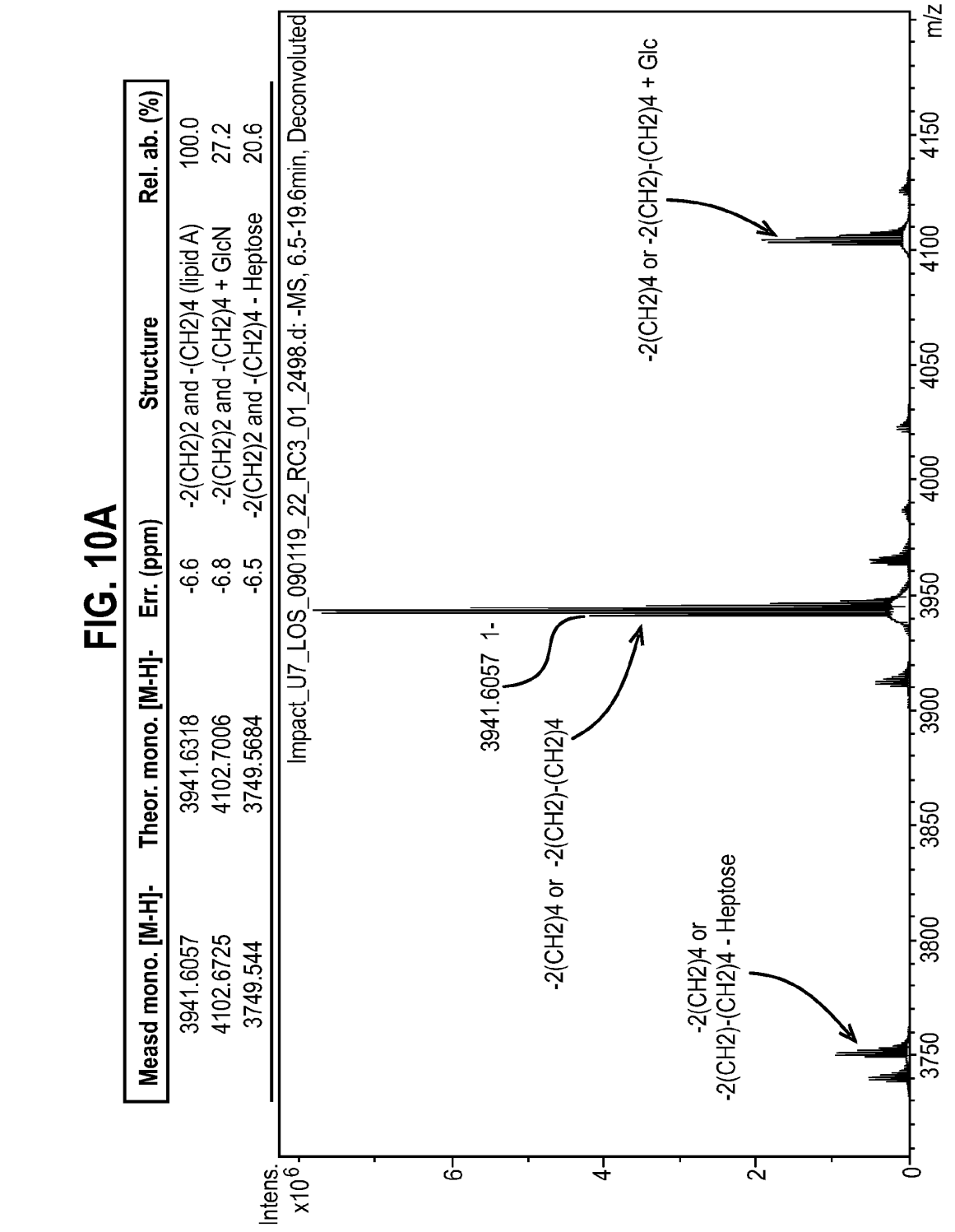


FIG. 9B

Exact Mass: 4053.7570

FIG. 10A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
3941.6057	3941.6318	-6.6	-2(CH2)2 and -(CH2)4 (lipid A)	100.0
4102.6725	4102.7006	-6.8	-2(CH2)2 and -(CH2)4 + GlcN	27.2
3749.544	3749.5684	-6.5	-2(CH2)2 and -(CH2)4 - Heptose	20.6



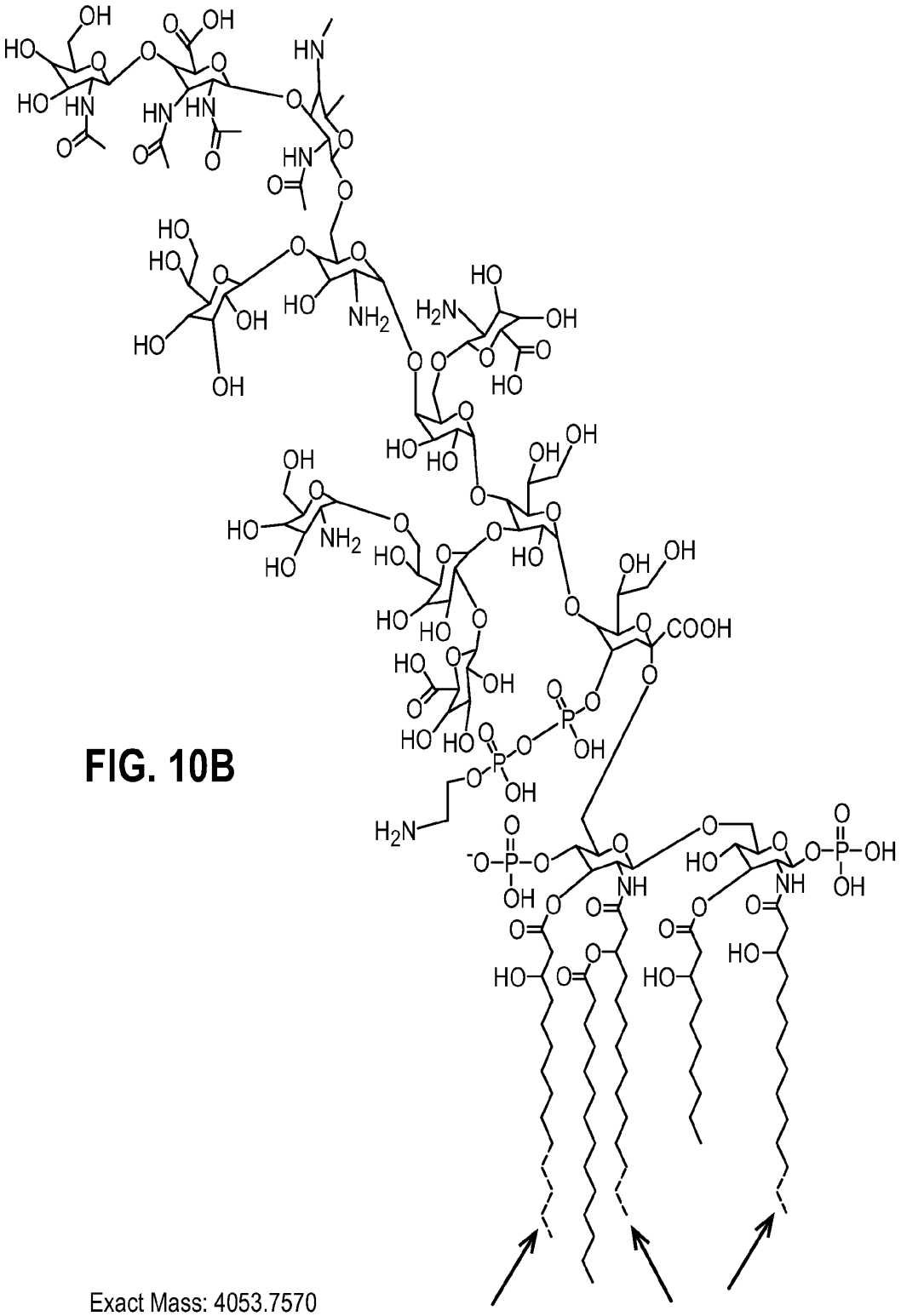
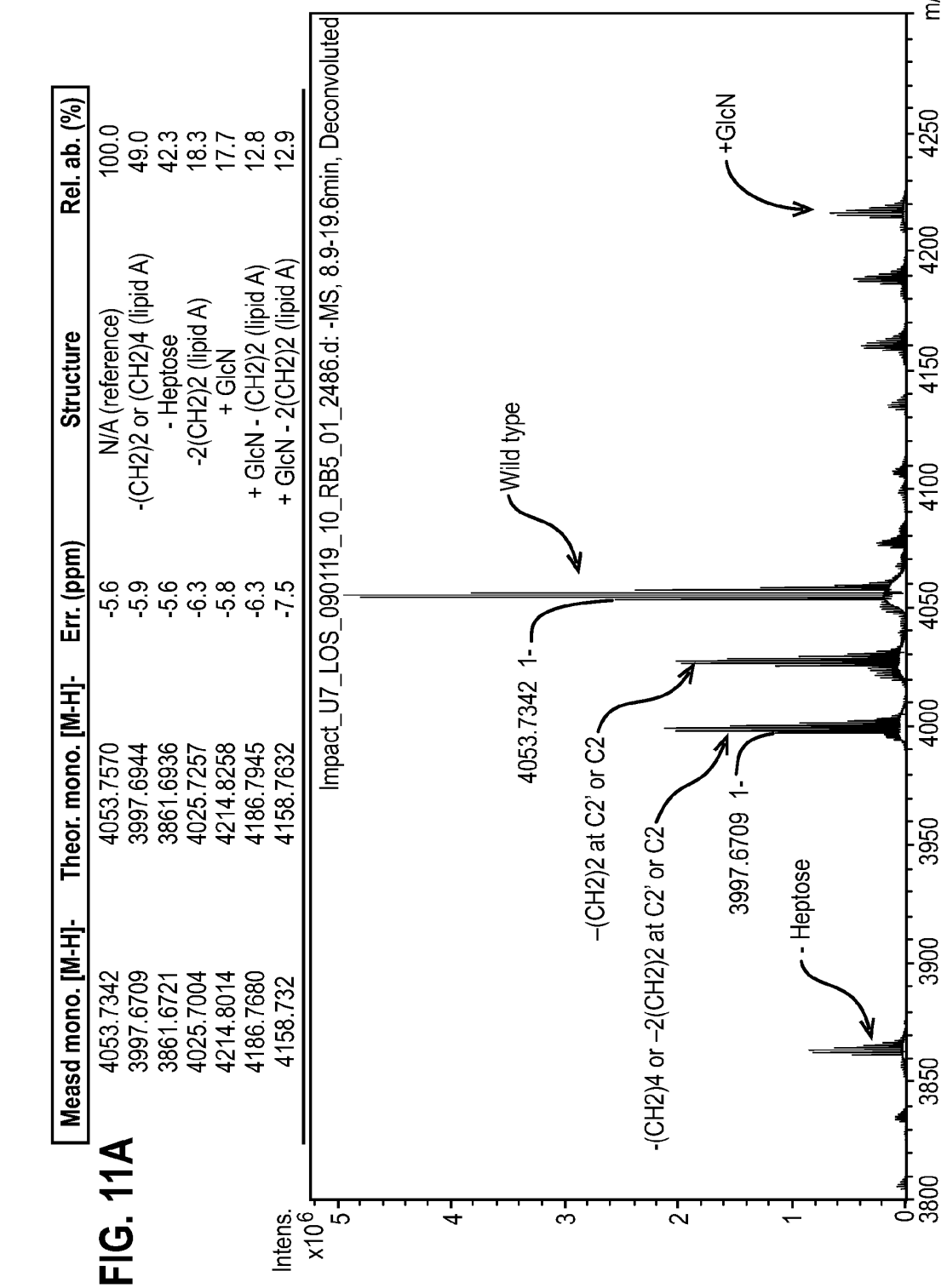


FIG. 10B

Exact Mass: 4053.7570

FIG. 11A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
4053.7342	4053.7570	-5.6	N/A (reference)	100.0
3997.6709	3997.6944	-5.9	-(CH2)2 or (CH2)4 (lipid A)	49.0
3861.6721	3861.6936	-5.6	- Heptose	42.3
4025.7004	4025.7257	-6.3	-2(CH2)2 (lipid A)	18.3
4214.8014	4214.8258	-5.8	+ GlcN	17.7
4186.7680	4186.7945	-6.3	+ GlcN - (CH2)2 (lipid A)	12.8
4158.732	4158.7632	-7.5	+ GlcN - 2(CH2)2 (lipid A)	12.9



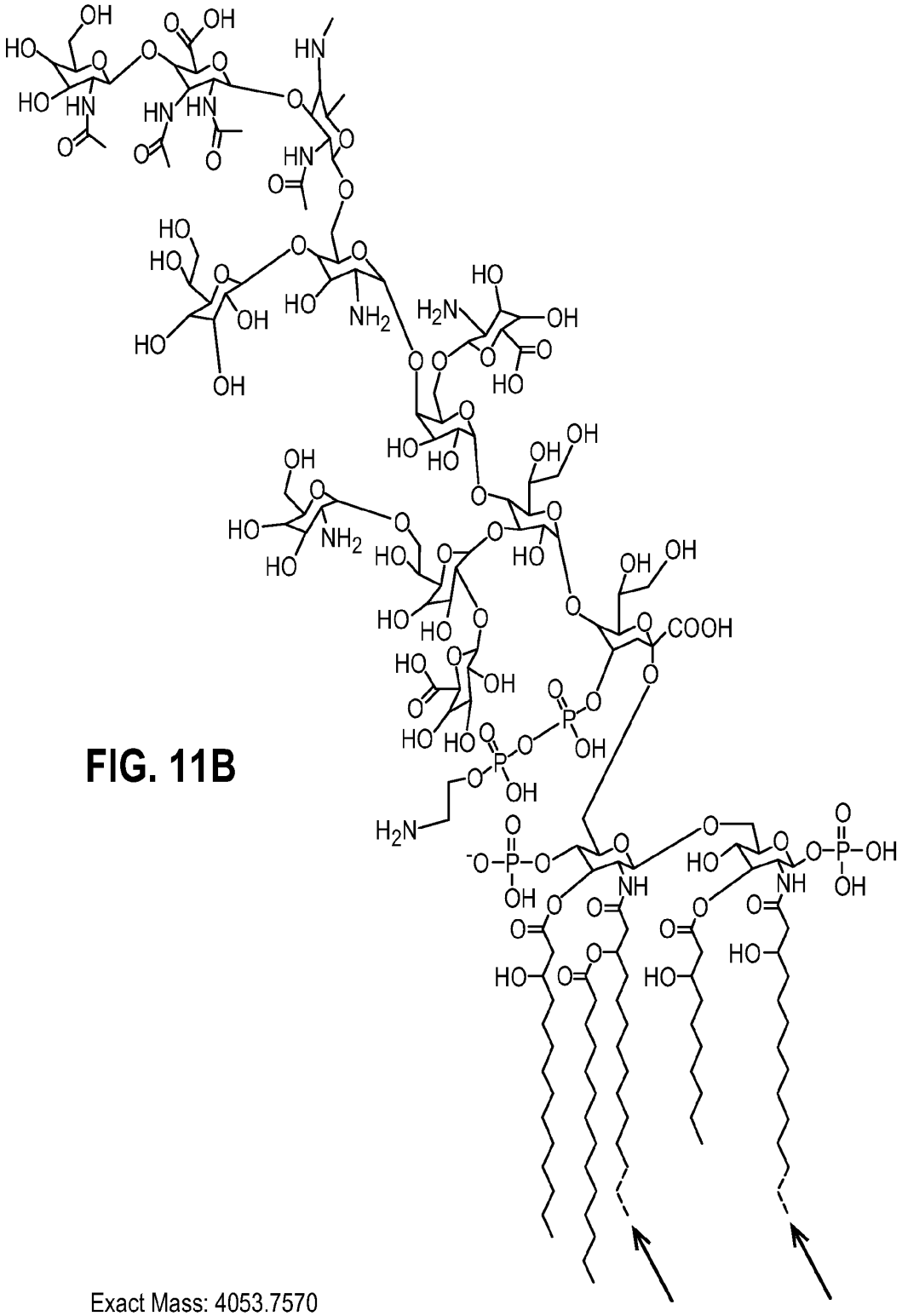


FIG. 11B

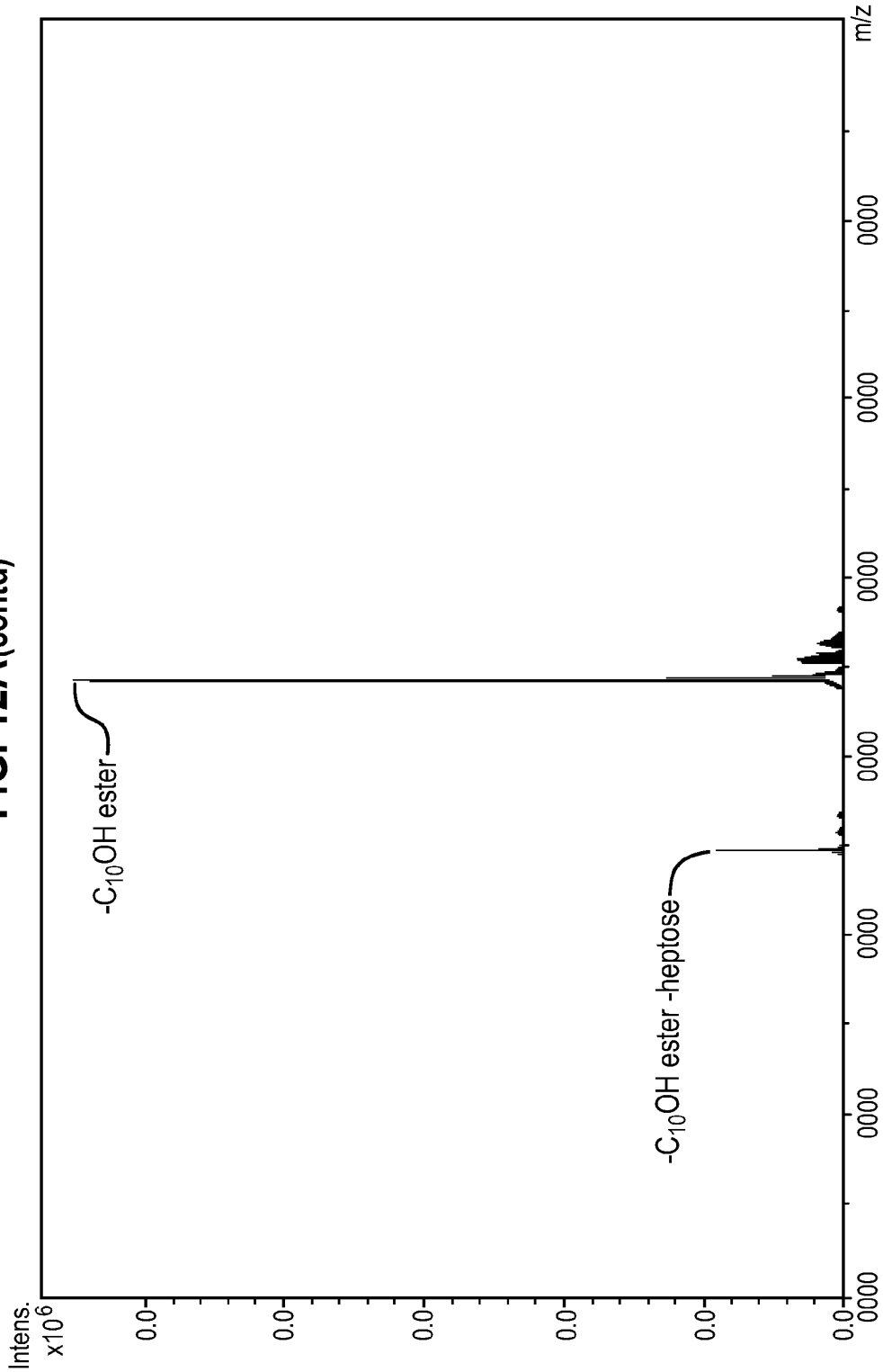
Exact Mass: 4053.7570

FIG. 12A

Measd mono. [M-H]-	Modifications	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
*	N/A (reference)	4053.7570		A	0.0
*	-PPEA	3850.7822		B	0.0
*	- C_4OH ester	3827.5637		C	0.0
*	-PPEA, - C_{14}OH ester	3624.5889		D	0.0
*	-Heptose	3861.6936		E	0.0
3883.5992	-C_{10}OH ester	3883.6263	-7.0	F	100.0
*	-PEA	3930.7485		G	0.0
*	-$(CH_2)_2$ (lipid A)	4025.7257		H	0.0
*	+$(CH_2)_2$ (lipid A)	4081.7883		I	0.0
*	+$(CH_2)_4$ (lipid A)	4109.8196		J	0.0
*	+GlcN	4214.8258		K	0.0
*	-(GlcNAcManXFucX)	3392.4764		L	0.0
*	-(GlcNAcManXFucX), -PPEA	3189.5015		M	0.0
3691.5368	-C_{10}OH ester,-Heptose	3691.5630	-7.1	N	17.0

* not detected

FIG. 12A(contd)



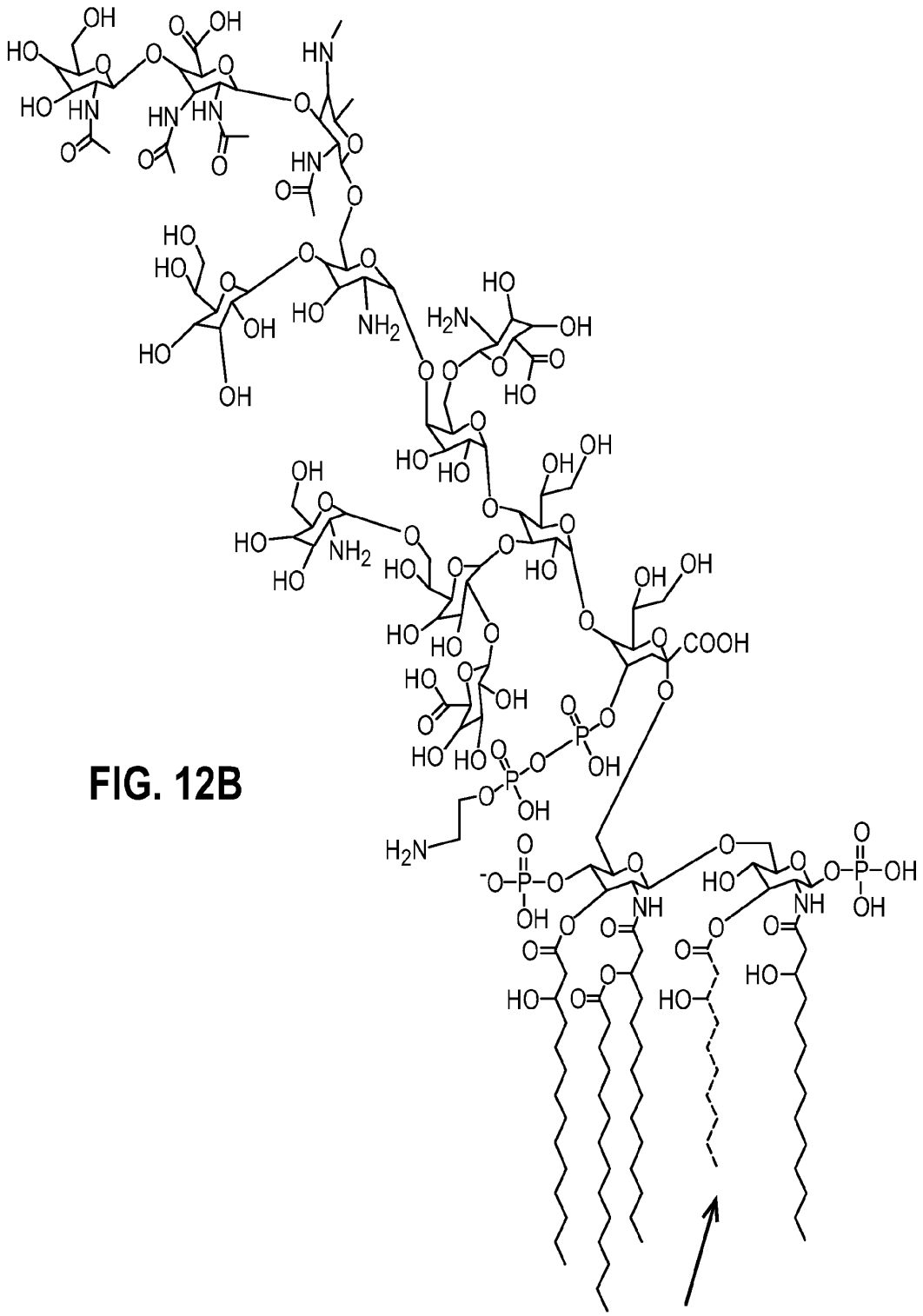
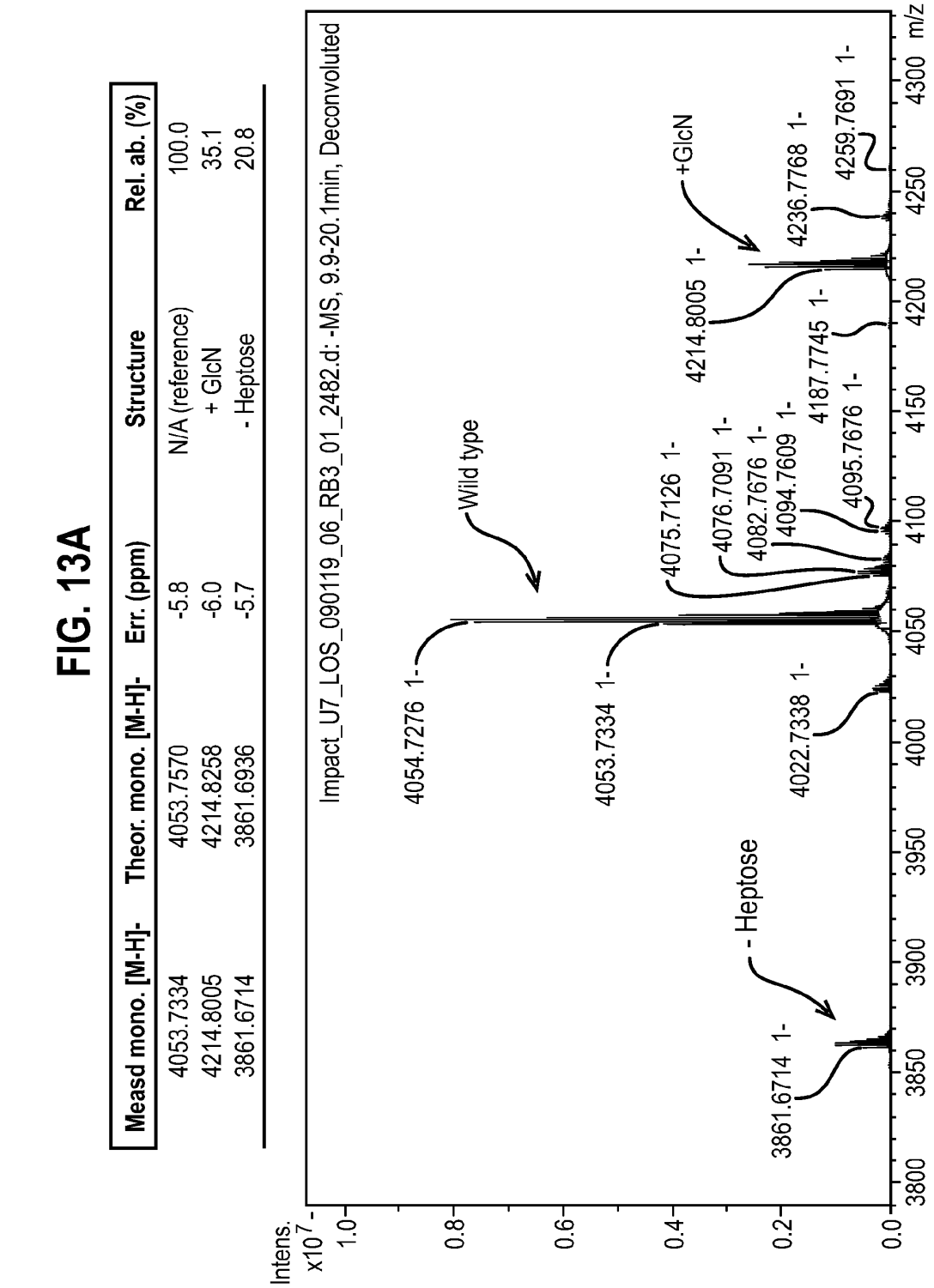


FIG. 12B

Exact Mass: 4053.7570

FIG. 13A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
4053.7334	4053.7570	-5.8	N/A (reference)	100.0
4214.8005	4214.8258	-6.0	+ GlcN	35.1
3861.6714	3861.6936	-5.7	- Heptose	20.8



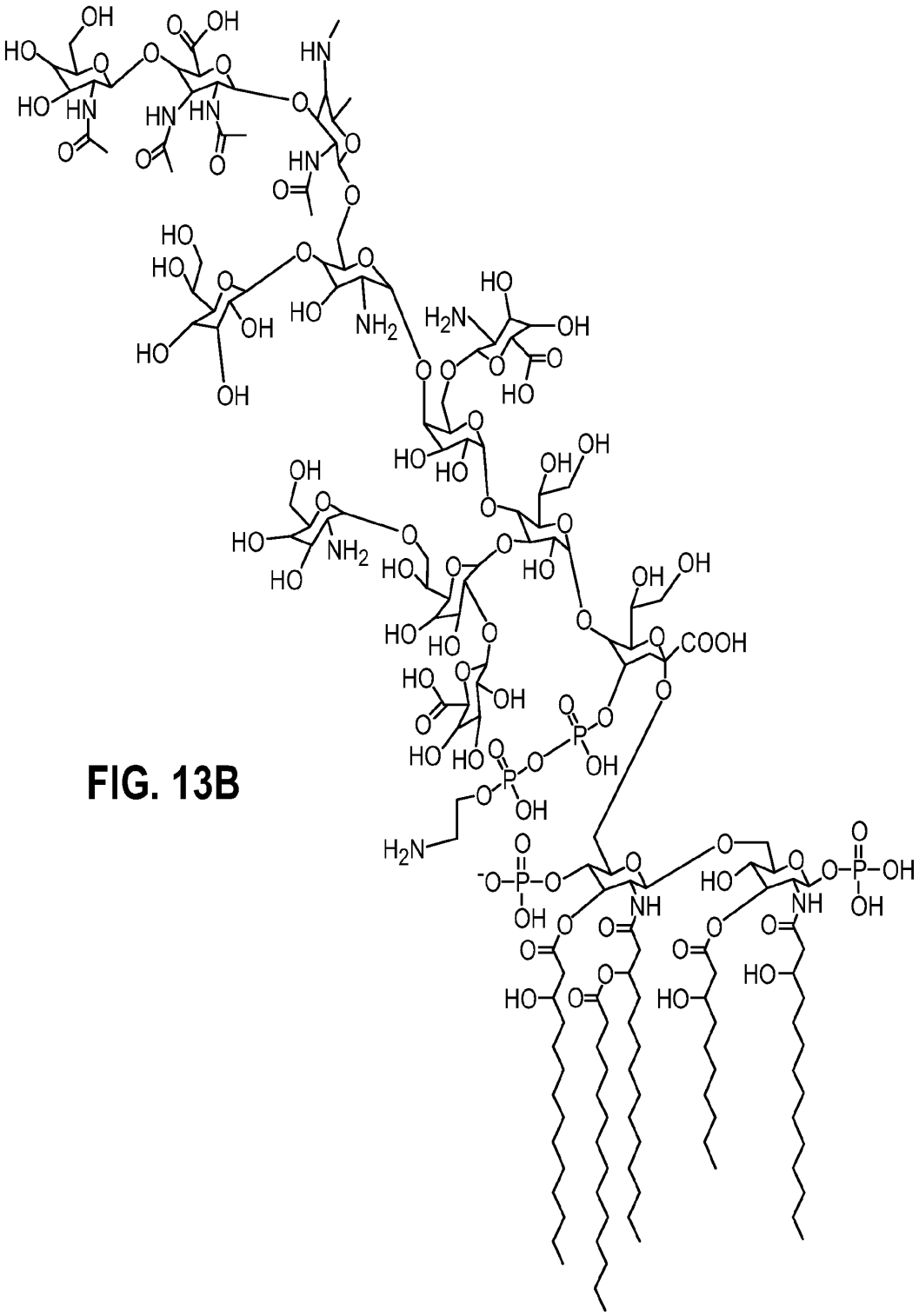


FIG. 13B

Exact Mass: 4053.7570

FIG. 14

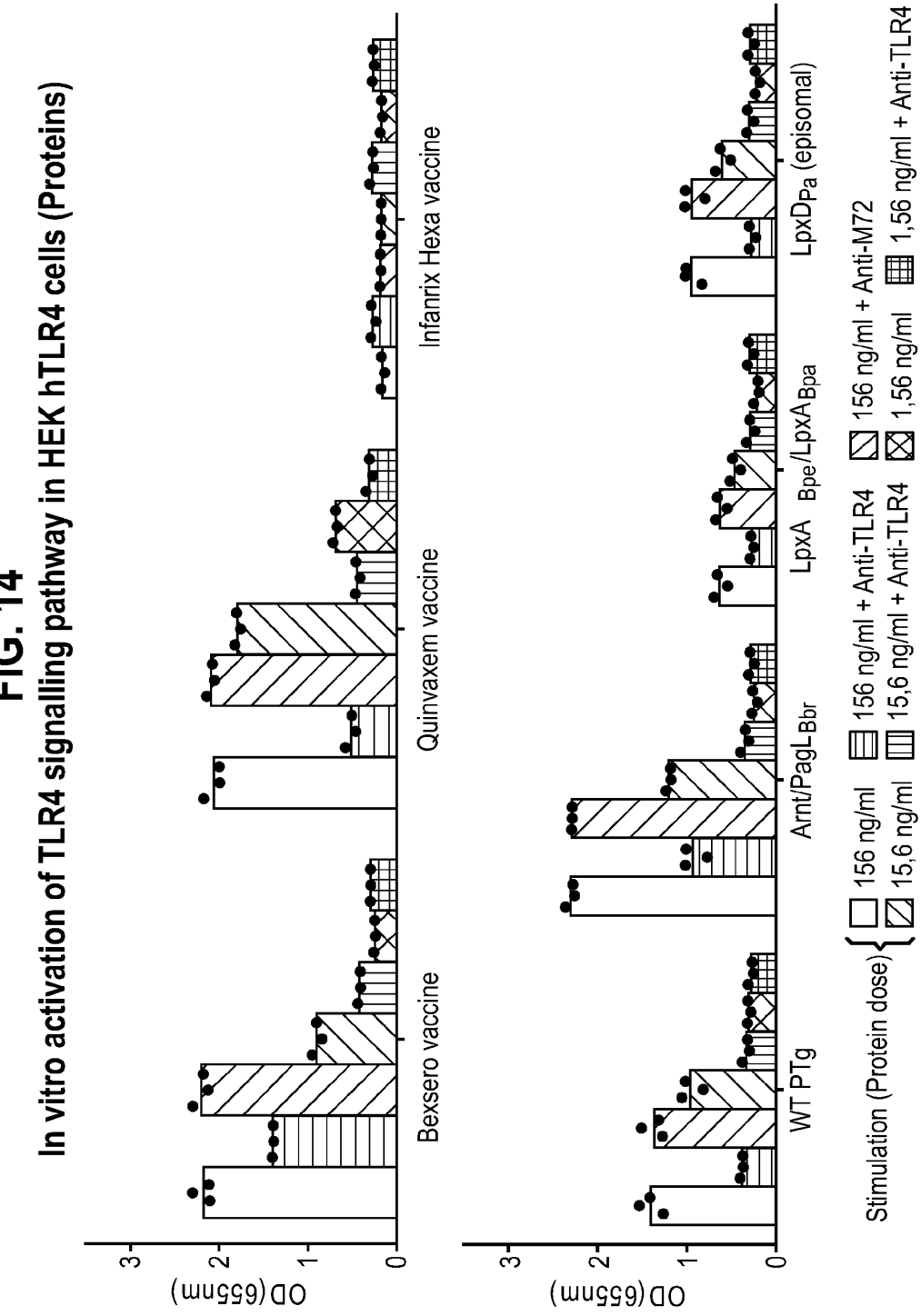

In vitro activation of TLR4 signalling pathway in HEK hTLR4 cells (Proteins)
OD (655nm)
0
1
2
3
Bexsero vaccine
Quinvaxem vaccine
Infanrix Hexa vaccine
WT PTg
Arnt/PagL Bbr
LpxA Bpe/LpxA Bpa
LpxD Pa (episomal)
Stimulation (Protein dose)
156 ng/ml
15,6 ng/ml
156 ng/ml + Anti-TLR4
15,6 ng/ml + Anti-TLR4
156 ng/ml + Anti-M72
1,56 ng/ml
1,56 ng/ml + Anti-TLR4

FIG. 15

In vitro activation of TLR4 signalling pathway in HEK-hTLR4 cells (LOS)

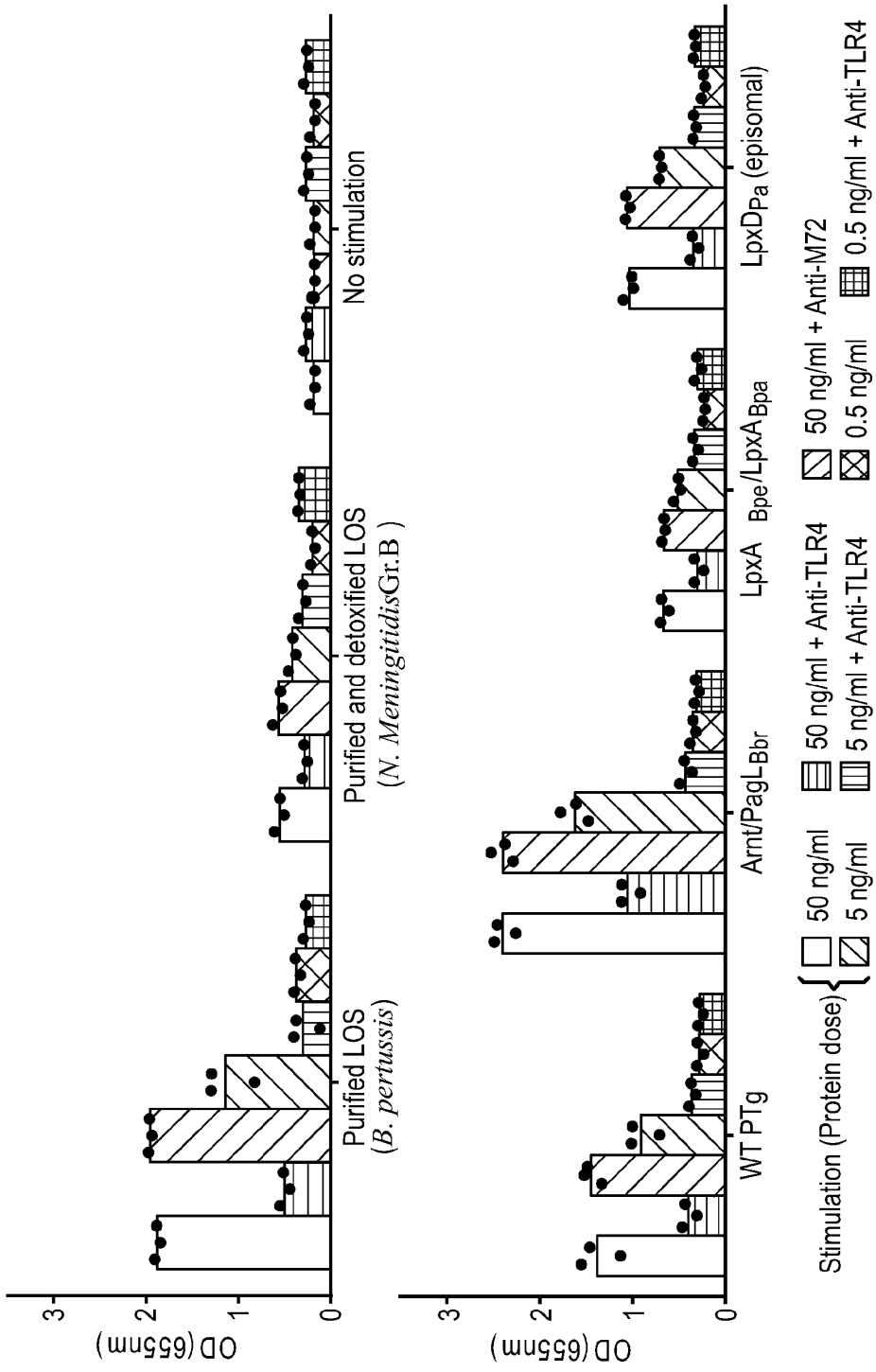


FIG. 16

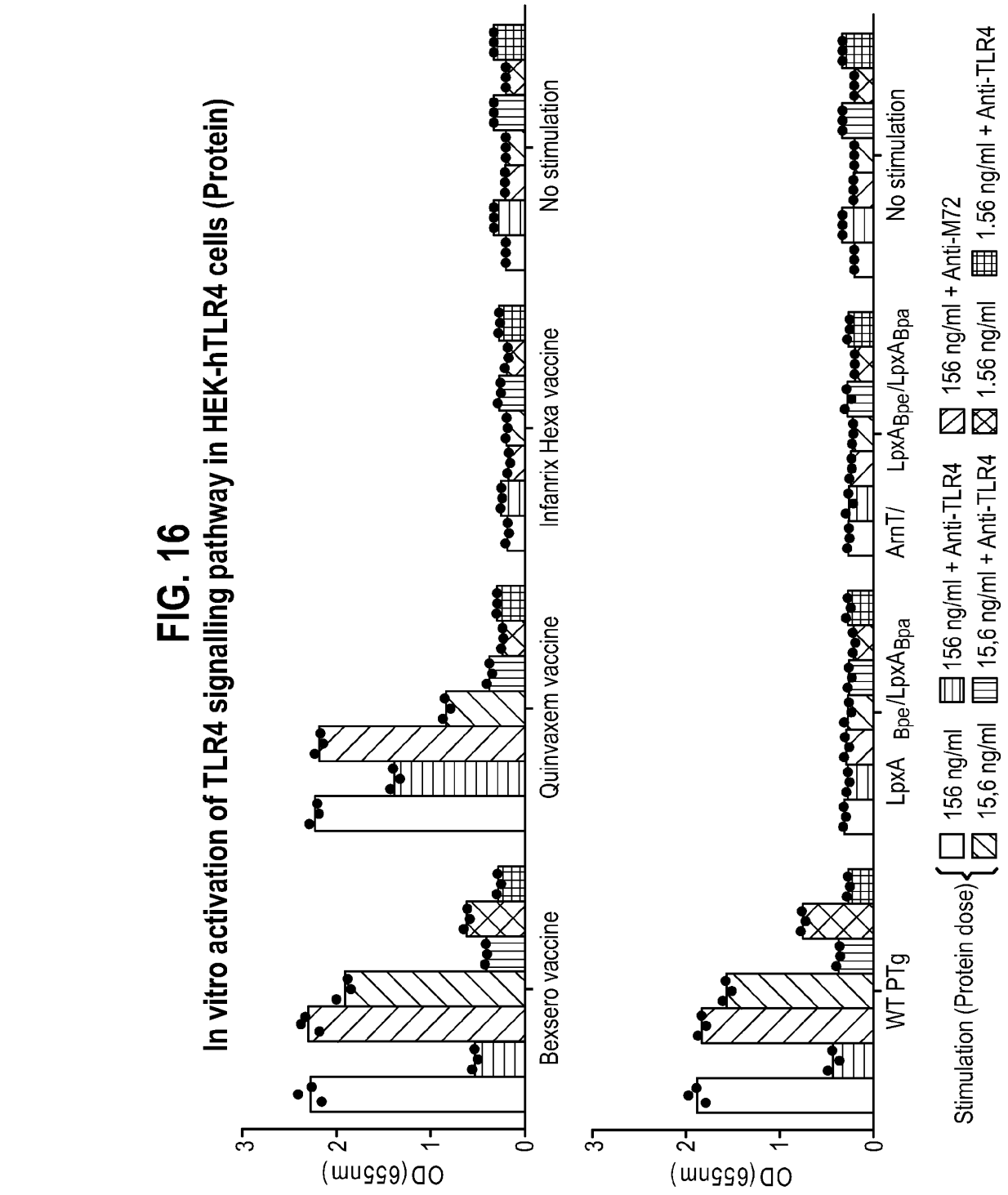

In vitro activation of TLR4 signalling pathway in HEK-hTLR4 cells (Protein)
OD (655nm)
0
1
2
3
Bexsero vaccine
Quinvaxem vaccine
Infanrix Hexa vaccine
No stimulation
WT PTg
LpxA Bpe/LpxABpa
ArnT/ LpxABpe/LpxABpa
No stimulation
Stimulation (Protein dose)
156 ng/ml
15,6 ng/ml
156 ng/ml + Anti-TLR4
15,6 ng/ml + Anti-TLR4
156 ng/ml + Anti-M72
1.56 ng/ml
1.56 ng/ml + Anti-TLR4

FIG. 16(contd)

In vitro activation of TLR4 signalling pathway in HEK-hTLR4 cells (Protein)

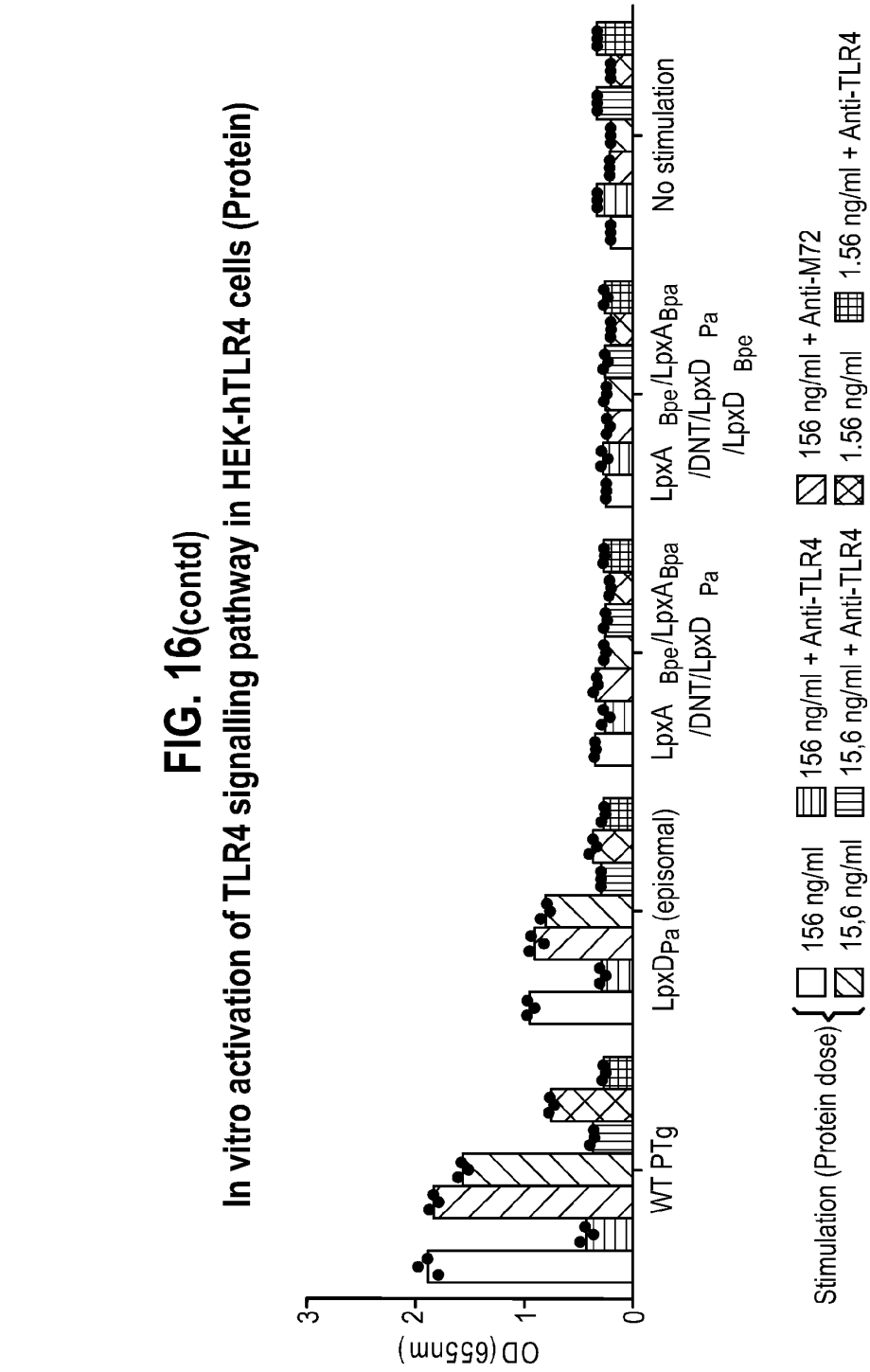


FIG. 17
In vitro activation of TLR4 signalling pathway in HEK-hTLR4 cells (LOS)

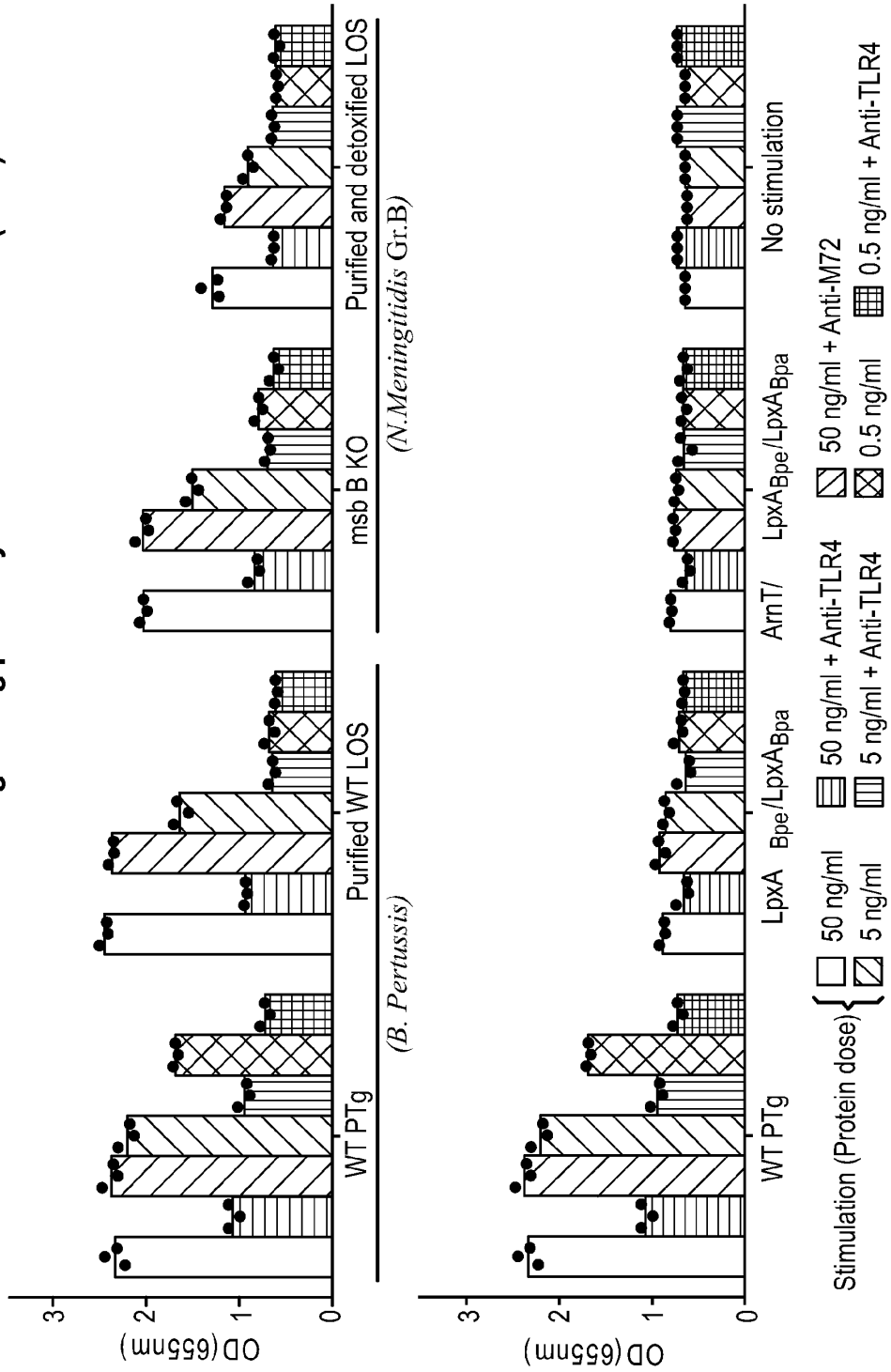


FIG. 17(contd)

In vitro activation of TLR4 signalling pathway in HEK-hTLR4 cells (LOS)

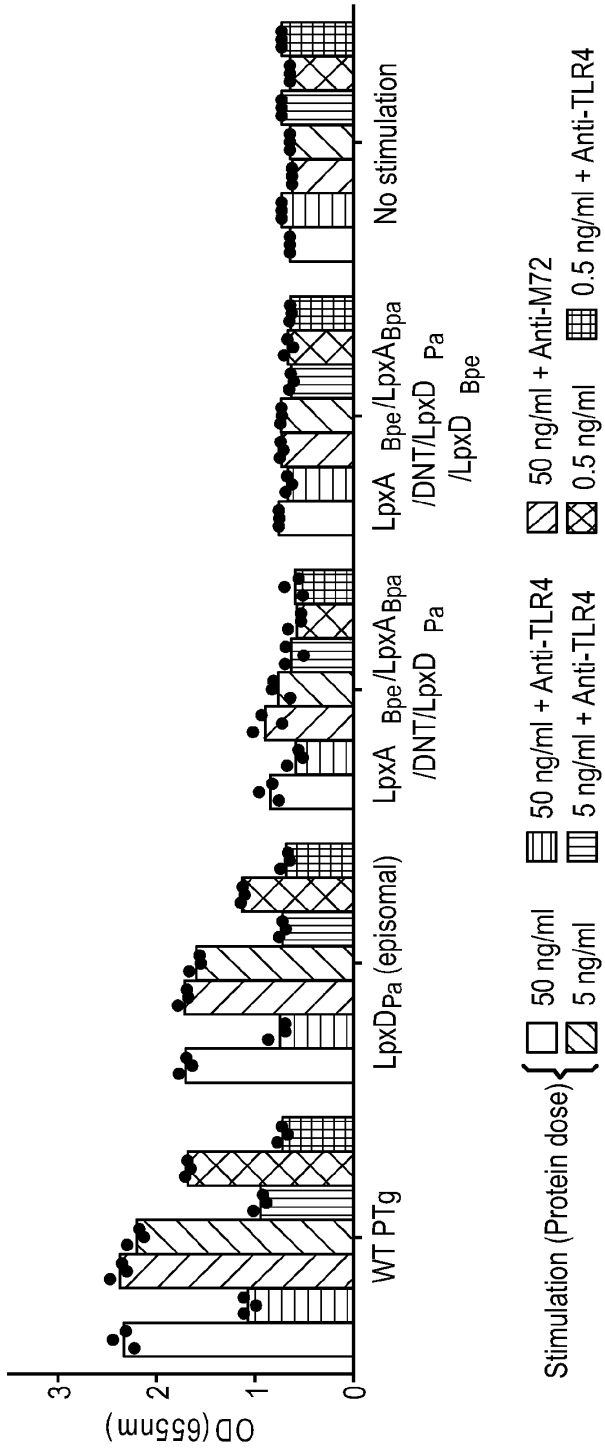
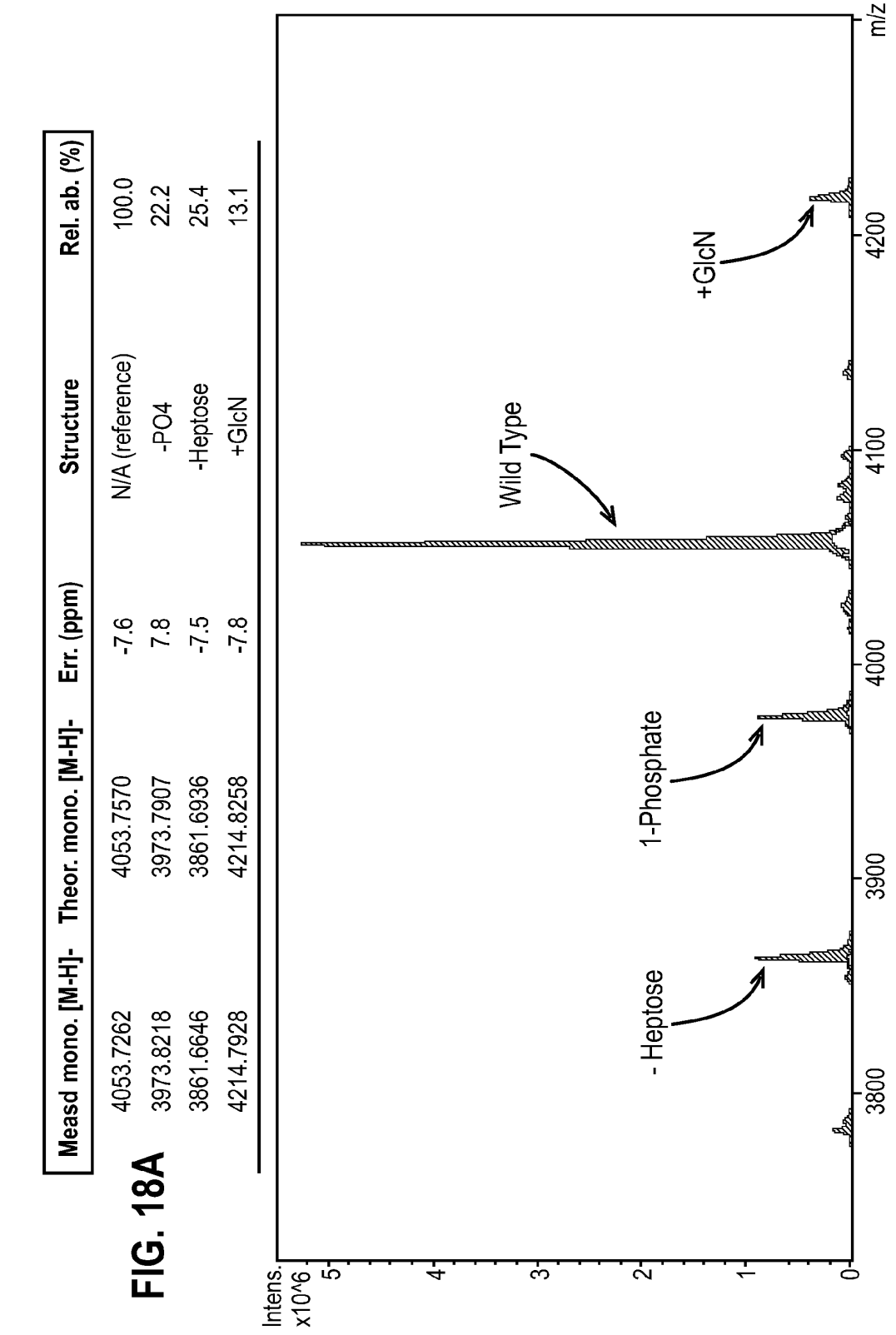


FIG. 18A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
4053.7262	4053.7570	-7.6	N/A (reference)	100.0
3973.8218	3973.7907	7.8	-PO4	22.2
3861.6646	3861.6936	-7.5	-Heptose	25.4
4214.7928	4214.8258	-7.8	+GlcN	13.1



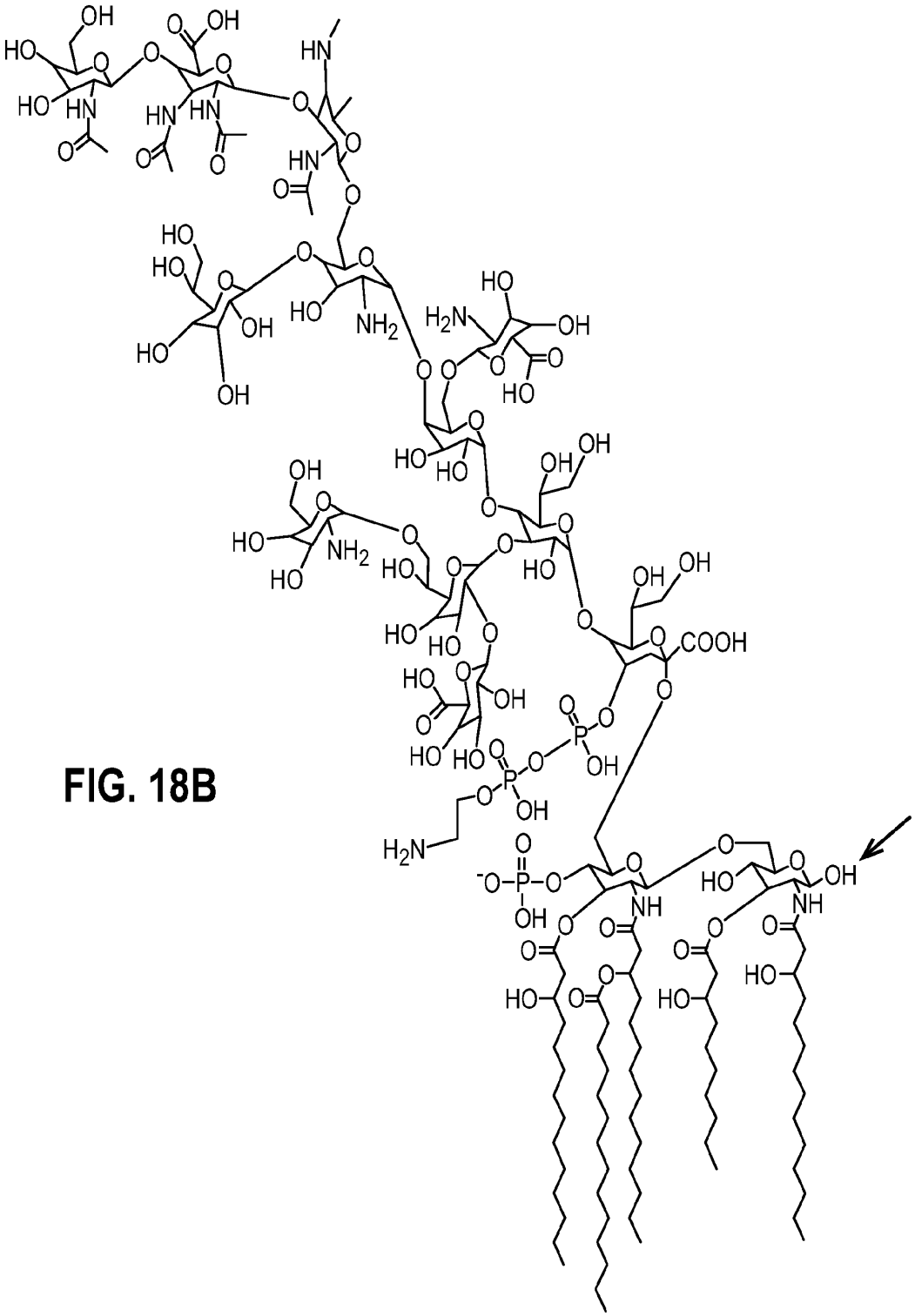
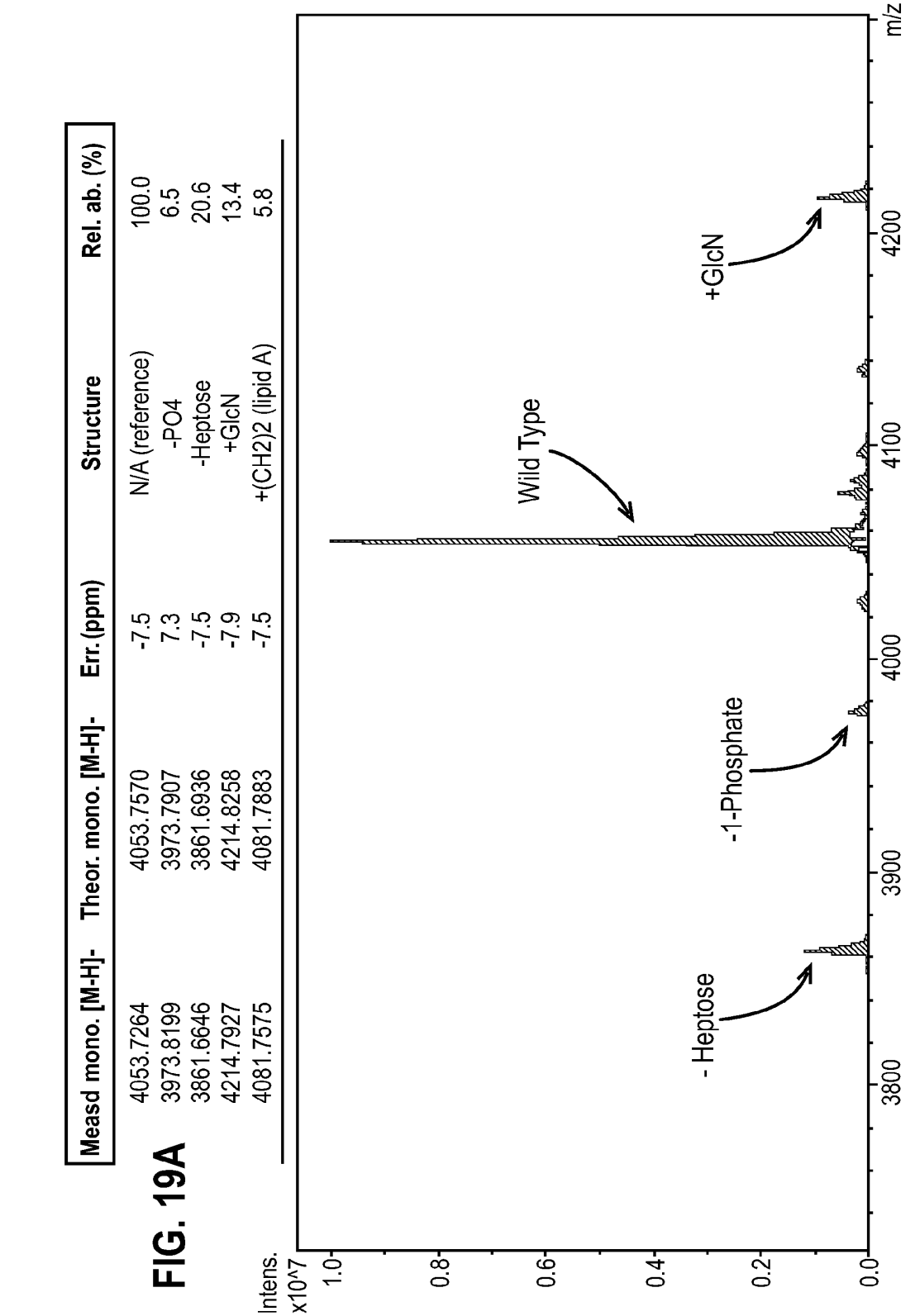


FIG. 18B

FIG. 19A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err. (ppm)	Structure	Rel. ab. (%)
4053.7264	4053.7570	-7.5	N/A (reference)	100.0
3973.8199	3973.7907	7.3	-PO4	6.5
3861.6646	3861.6936	-7.5	-Heptose	20.6
4214.7927	4214.8258	-7.9	+GlcN	13.4
4081.7575	4081.7883	-7.5	+(CH2)2 (lipid A)	5.8



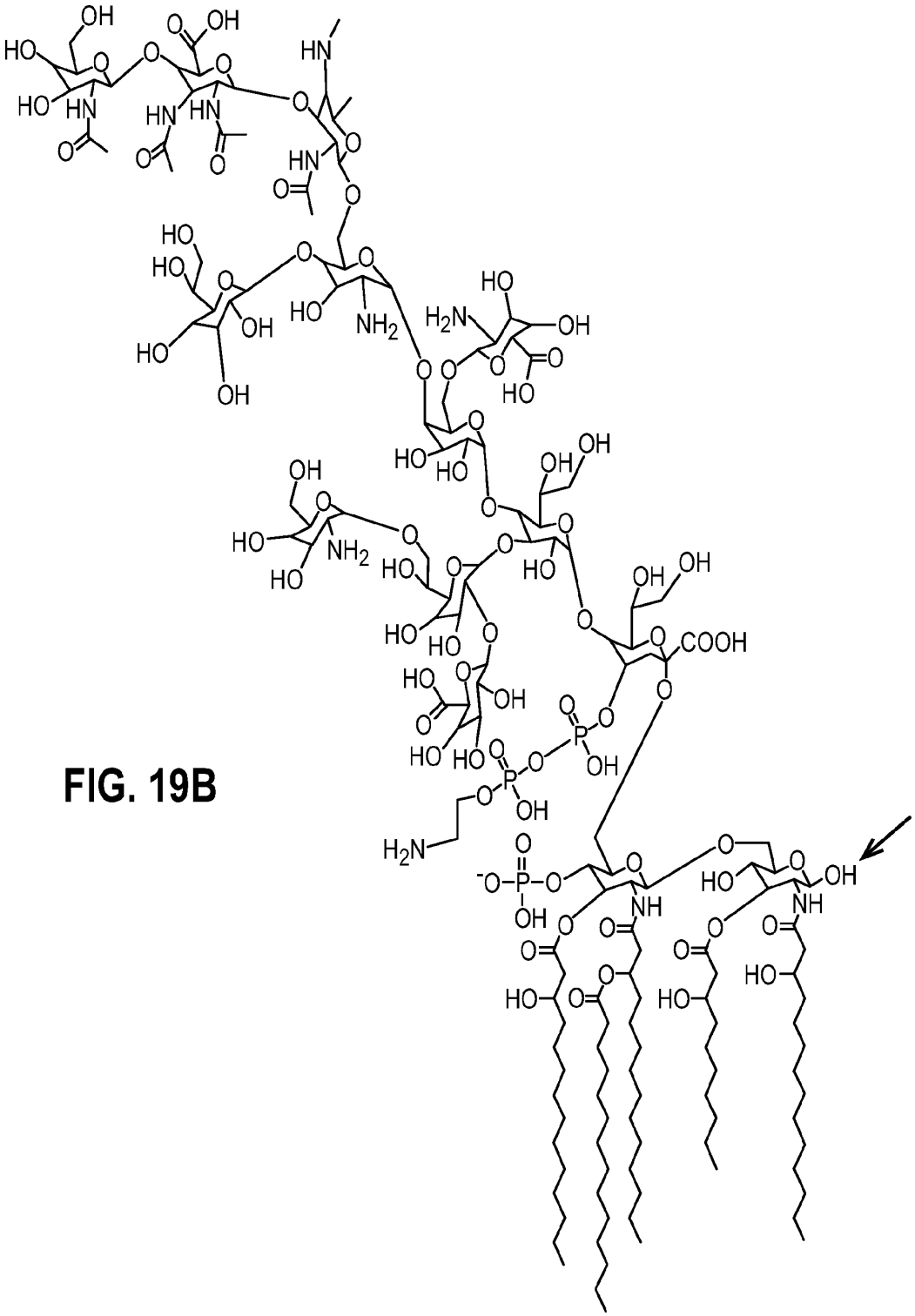
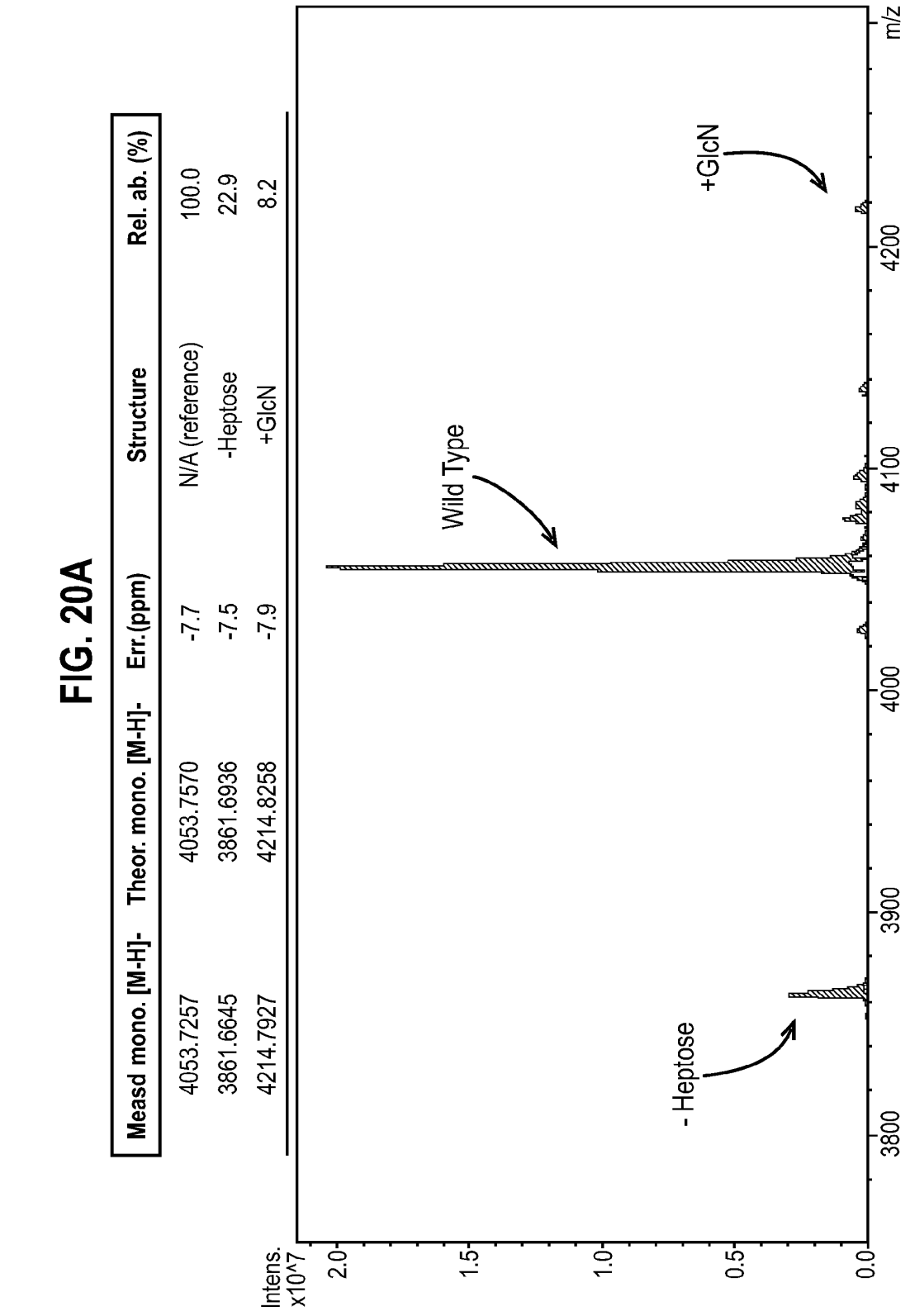


FIG. 19B

FIG. 20A

Measd mono. [M-H]-	Theor. mono. [M-H]-	Err.(ppm)	Structure	Rel. ab. (%)
4053.7257	4053.7570	-7.7	N/A (reference)	100.0
3861.6645	3861.6936	-7.5	-Heptose	22.9
4214.7927	4214.8258	-7.9	+GlcN	8.2



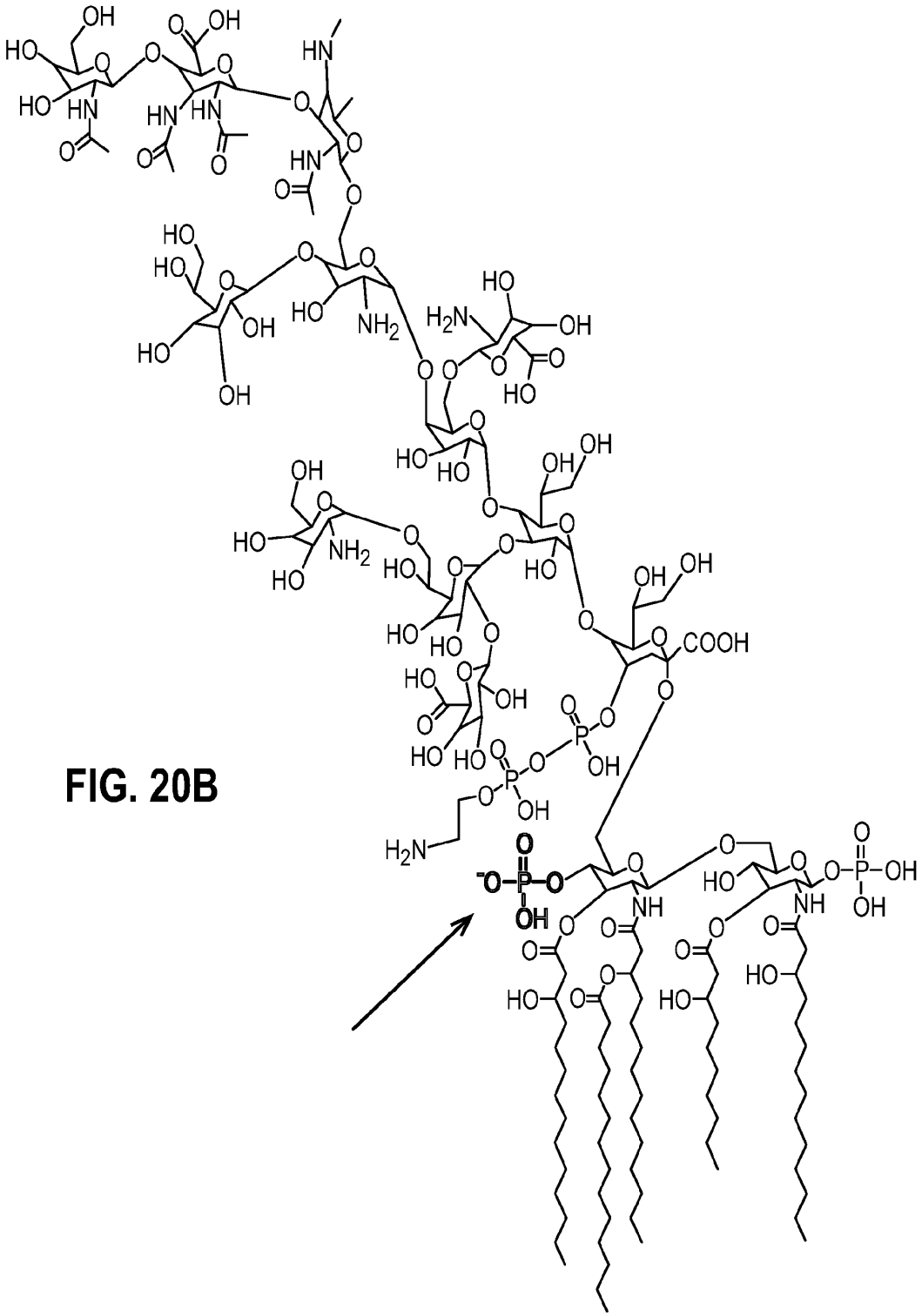


FIG. 20B

FIG. 21

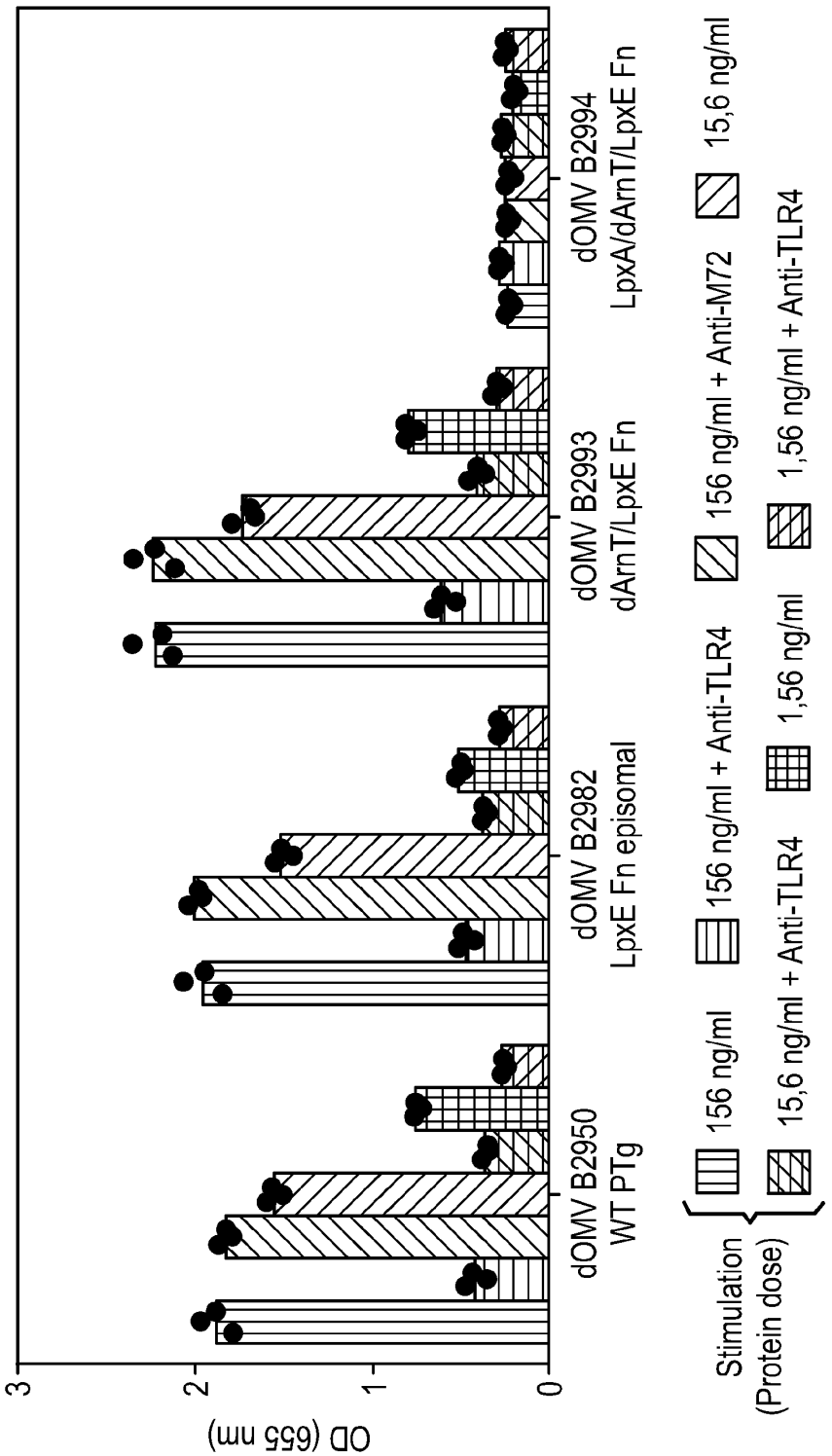

OD (655 nm)
0
1
2
3
dOMV B2950
WT PTg
dOMV B2982
LpxE Fn episomal
dOMV B2993
dArnT/LpxE Fn
dOMV B2994
LpxA/dArnT/LpxE Fn
Stimulation
(Protein dose)
156 ng/ml
156 ng/ml + Anti-TLR4
156 ng/ml + Anti-M72
15,6 ng/ml
15,6 ng/ml + Anti-TLR4
1,56 ng/ml
1,56 ng/ml + Anti-TLR4

FIG. 22

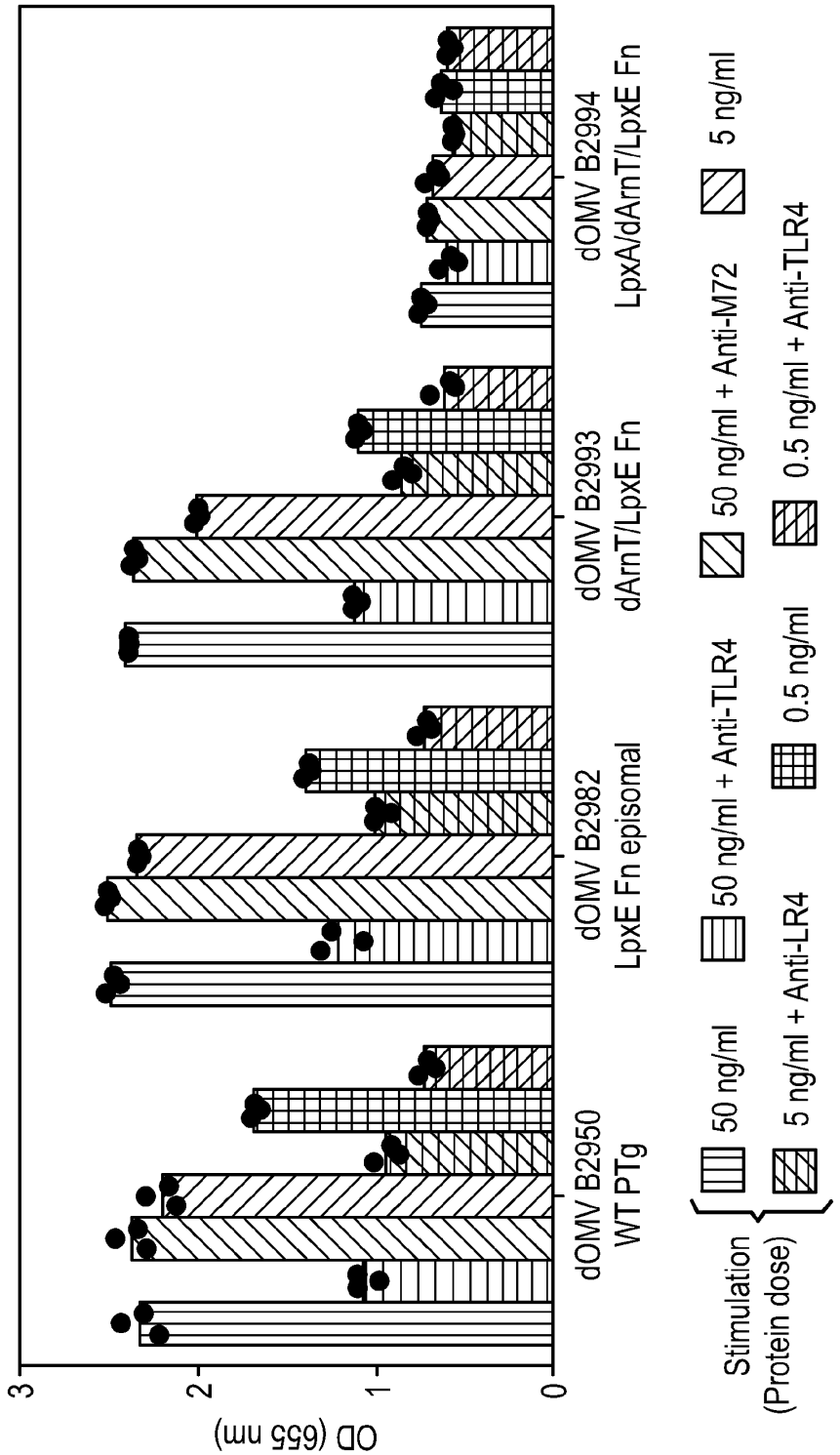

OD (655 nm)
0
1
2
3
dOMV B2950
WT PTg
dOMV B2982
LpxE Fn episomal
dOMV B2993
dArnT/LpxE Fn
dOMV B2994
LpxA/dArnT/LpxE Fn
Stimulation
(Protein dose)
50 ng/ml
5 ng/ml + Anti-LR4
50 ng/ml + Anti-TLR4
0.5 ng/ml
50 ng/ml + Anti-M72
0.5 ng/ml + Anti-TLR4
5 ng/ml

FIG. 23

Individual results and geometric mean with 95% CI

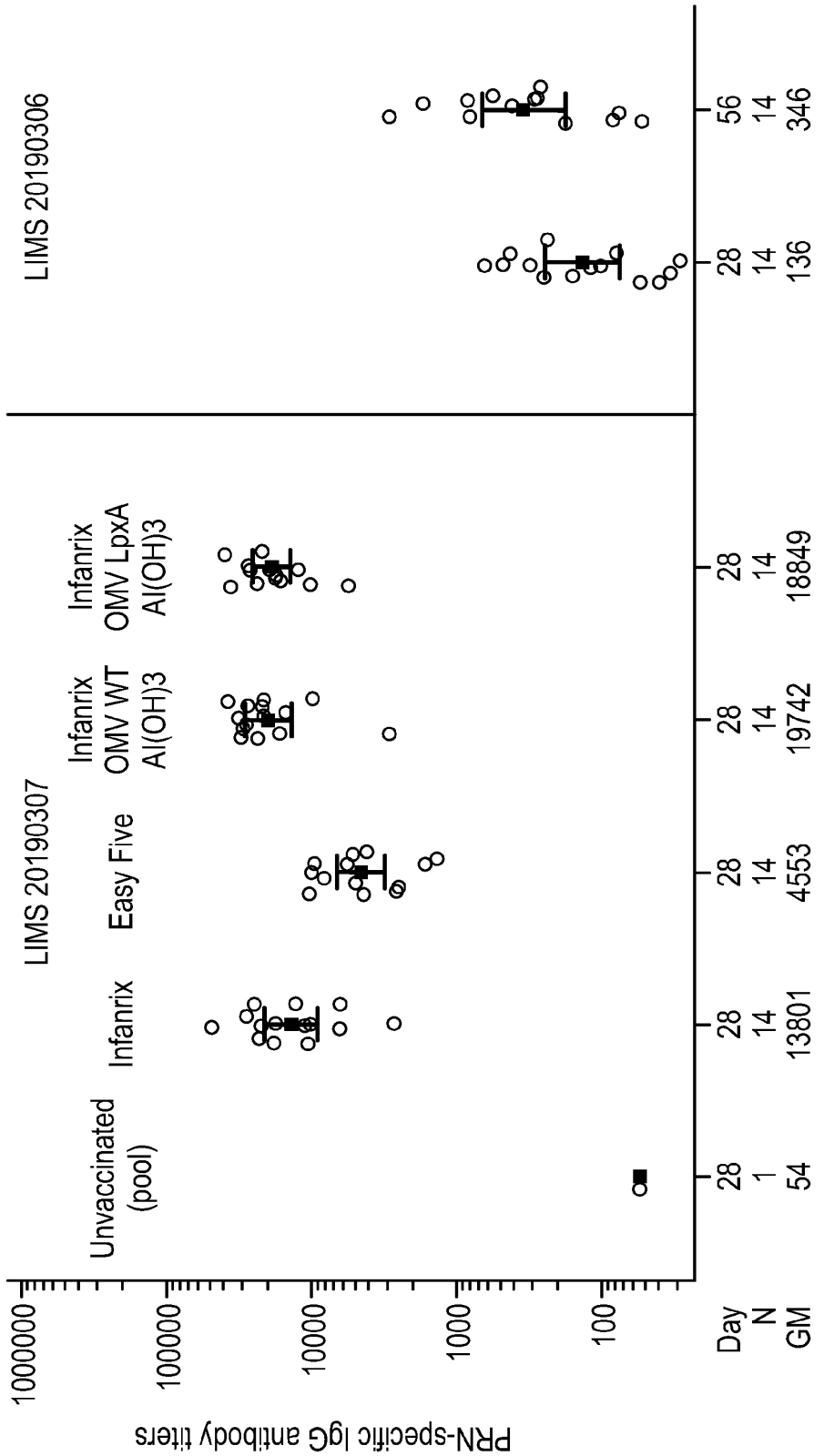
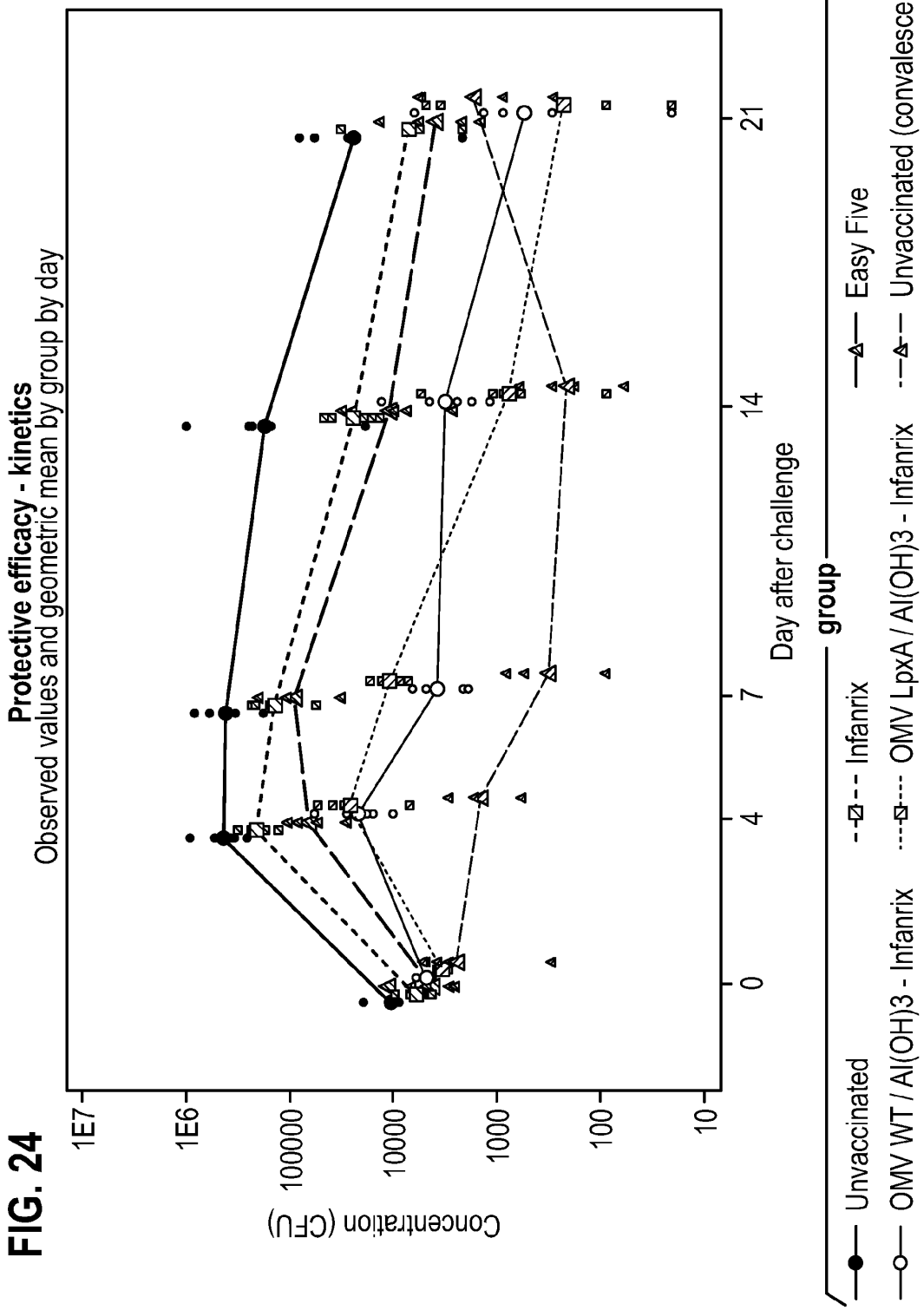


FIG. 24


Protective efficacy - kinetics
Observed values and geometric mean by group by day
Concentration (CFU)
1E7
1E6
100000
10000
1000
100
10
0
4
7
14
21
Day after challenge
group
Unvaccinated
Infanrix
Easy Five
OMV WT / Al(OH)3 - Infanrix
OMV LpxA / Al(OH)3 - Infanrix
Unvaccinated (convalescent mice)

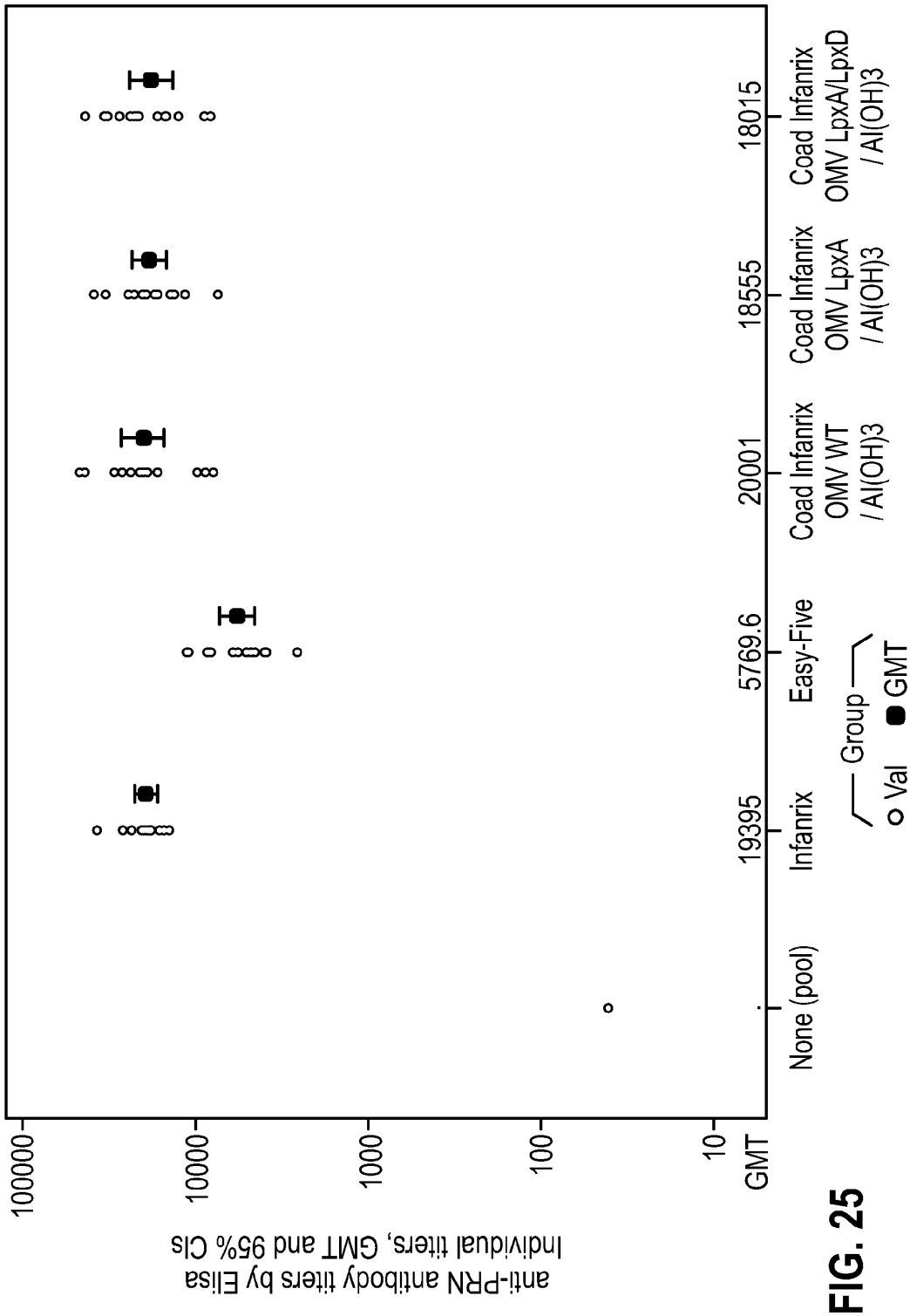
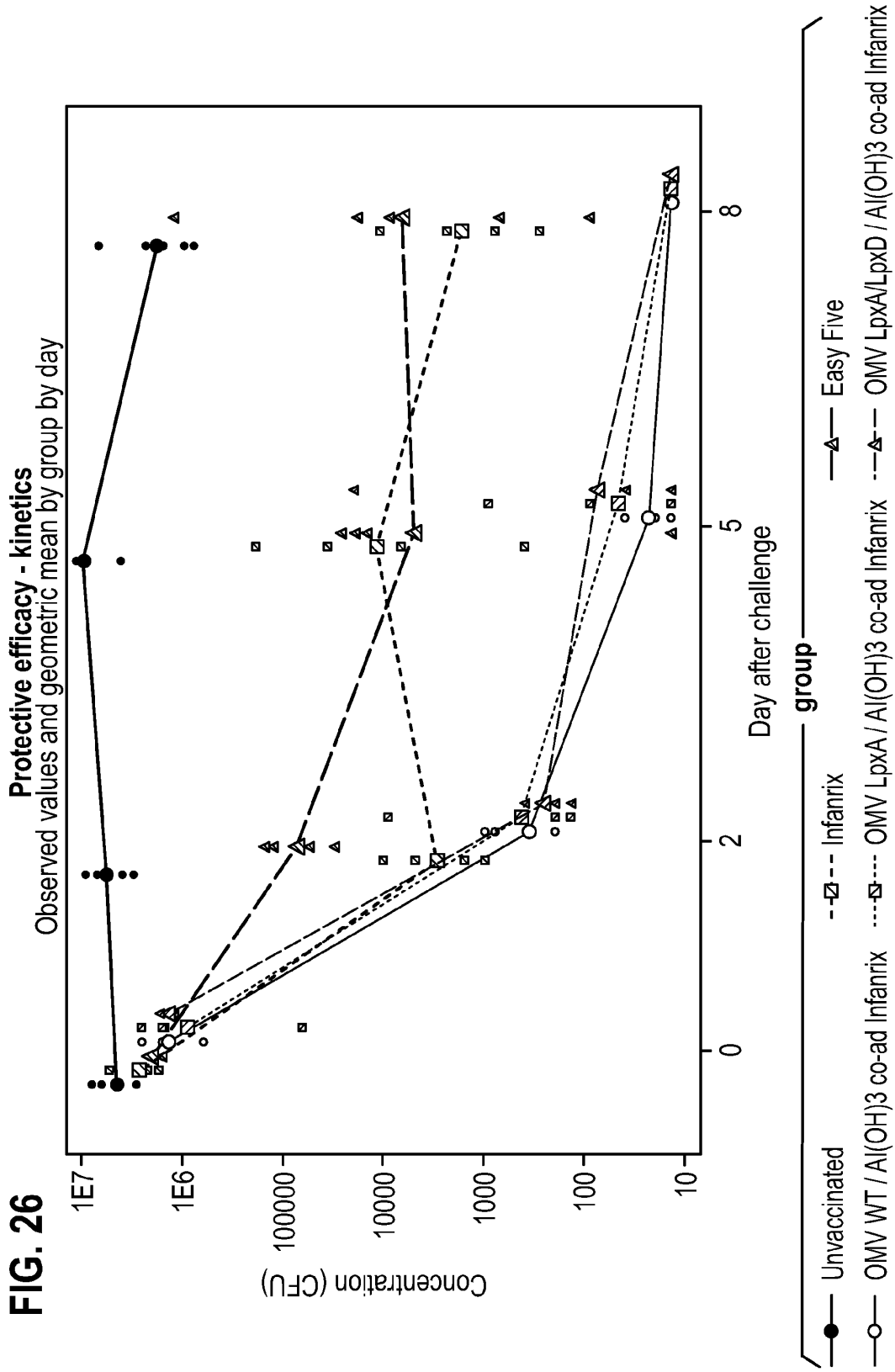

anti-PRN antibody titers by Elisa
Individual titers, GMT and 95% CIs
100000
10000
1000
100
10
GMT
None (pool)
Infanrix
Easy-Five
Coad Infanrix OMV WT /AI(OH)3
Coad Infanrix OMV LpxA /AI(OH)3
Coad Infanrix OMV LpxA/LpxD /AI(OH)3
19395
5769.6
20001
18555
18015
Group
Val
GMT

FIG. 25

FIG. 26


Protective efficacy - kinetics
Observed values and geometric mean by group by day
Concentration (CFU)
1E7
1E6
100000
10000
1000
100
10
0
2
5
8
Day after challenge
group
Unvaccinated
Infanrix
Easy Five
OMV WT / Al(OH)3 co-ad Infanrix
OMV LpxA / Al(OH)3 co-ad Infanrix
OMV LpxA/LpxD / Al(OH)3 co-ad Infanrix

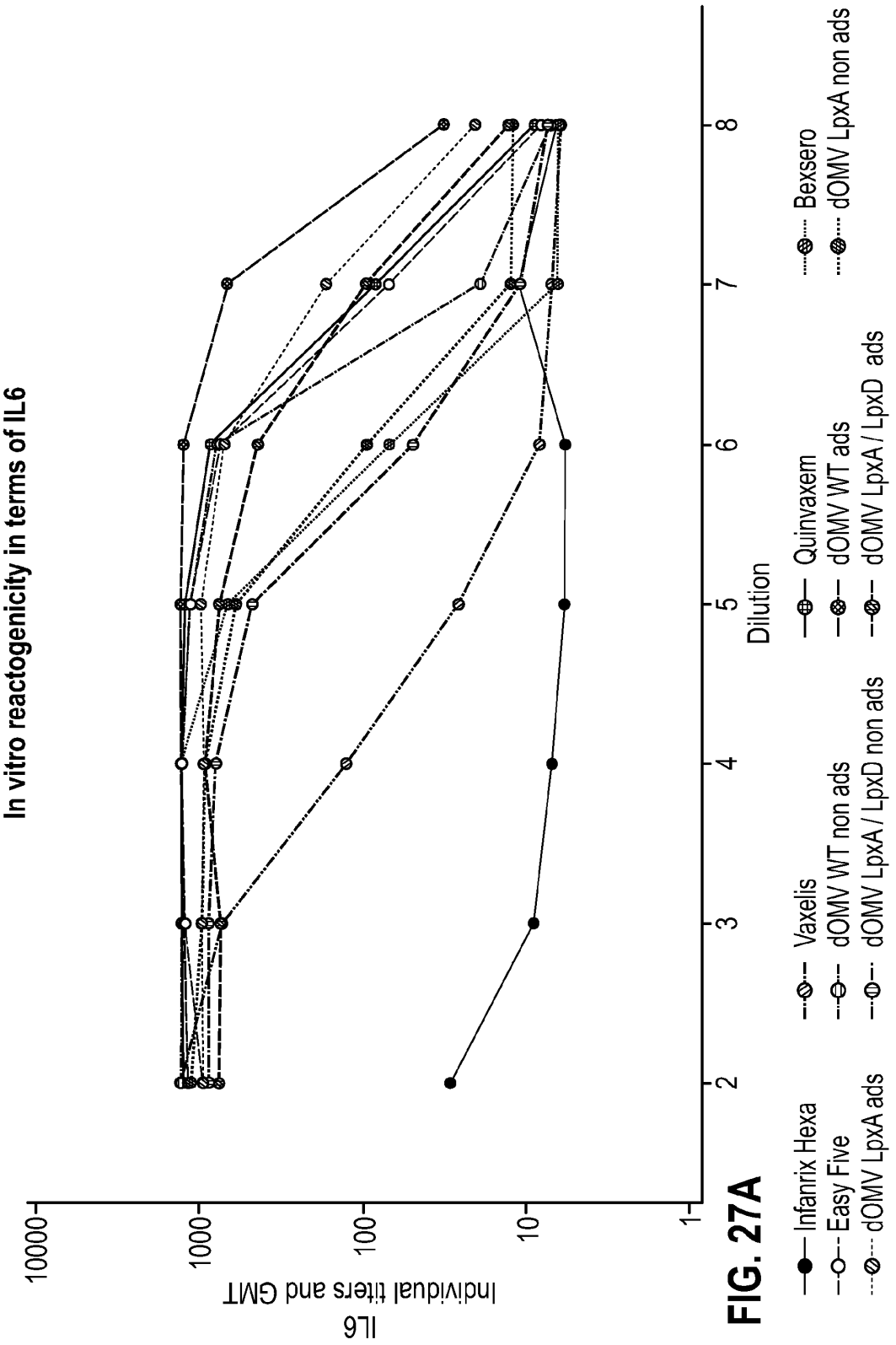

In vitro reactogenicity in terms of IL6
IL6
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Easy Five
dOMV LpxA ads
Vaxelis
dOMV WT non ads
dOMV LpxA / LpxD non ads
Quinvaxem
dOMV WT ads
dOMV LpxA / LpxD ads
Bexsero
dOMV LpxA non ads

FIG. 27A

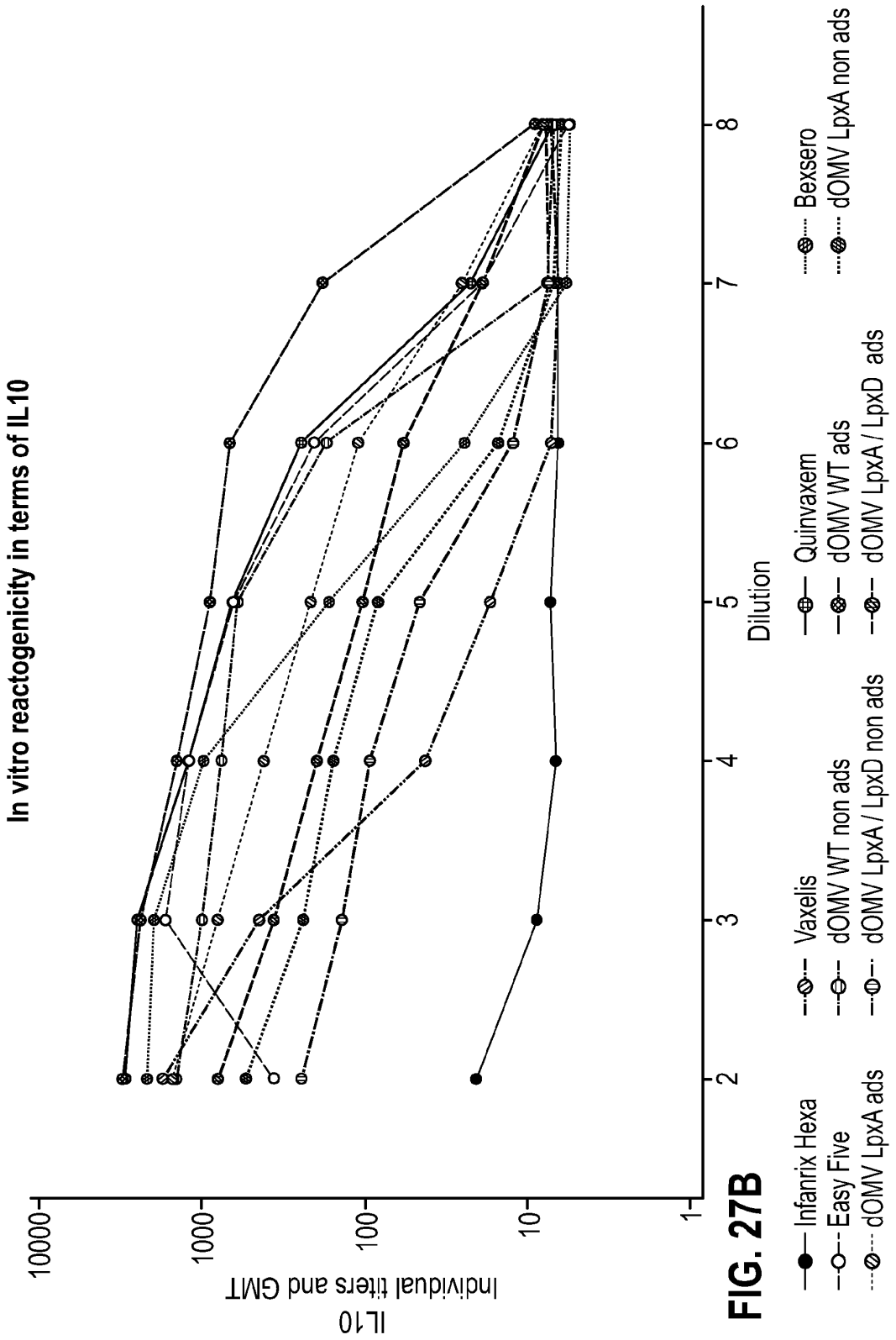

In vitro reactogenicity in terms of IL10
IL10
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Easy Five
dOMV LpxA ads
Vaxelis
dOMV WT non ads
dOMV LpxA / LpxD non ads
Quinvaxem
dOMV WT ads
dOMV LpxA / LpxD ads
Bexsero
dOMV LpxA non ads

FIG. 27B

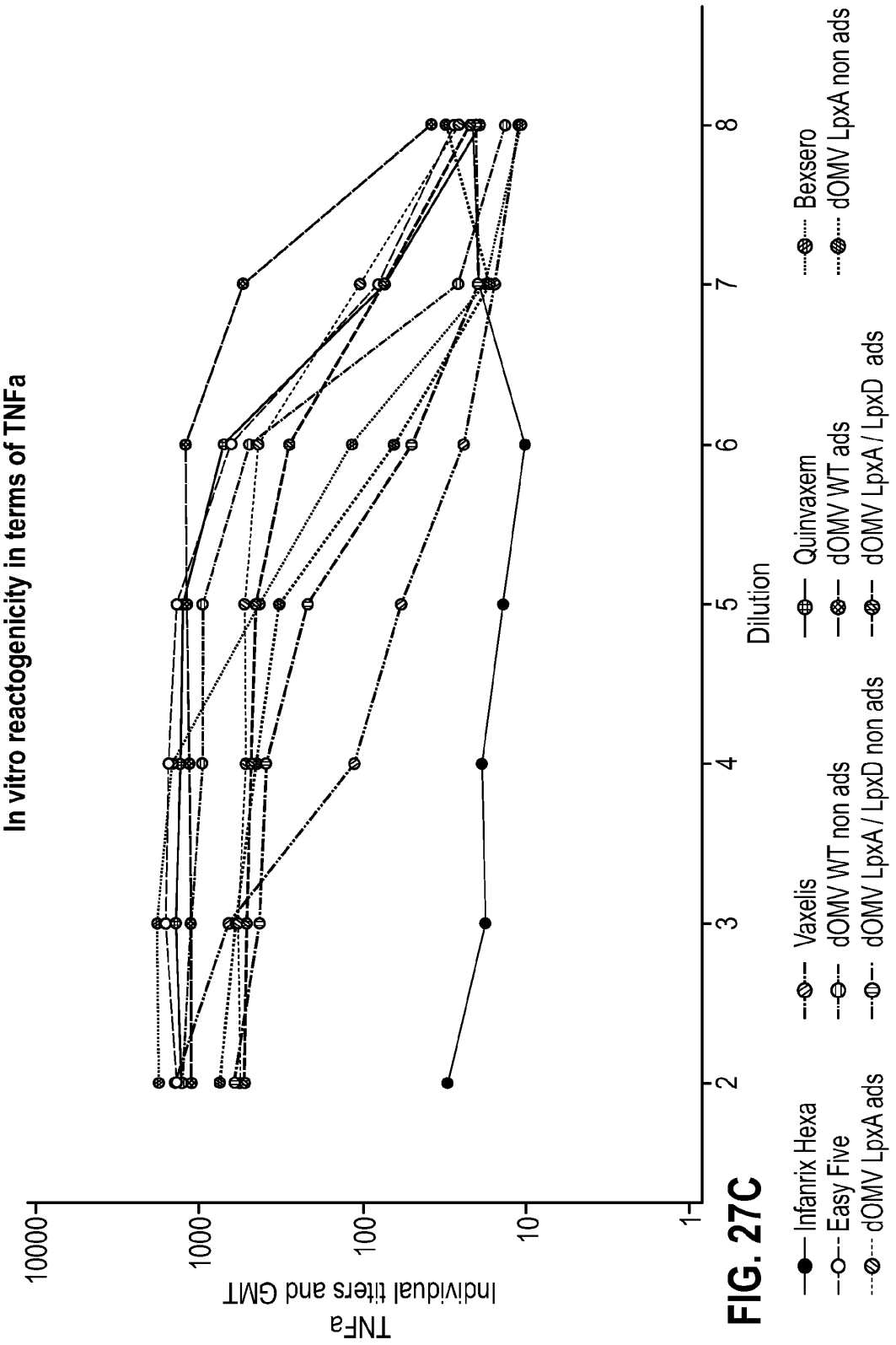

In vitro reactogenicity in terms of TNFa
TNFa
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Easy Five
dOMV LpxA ads
Vaxelis
dOMV WT non ads
dOMV LpxA / LpxD non ads
Quinvaxem
dOMV WT ads
dOMV LpxA / LpxD ads
Bexsero
dOMV LpxA non ads

FIG. 27C

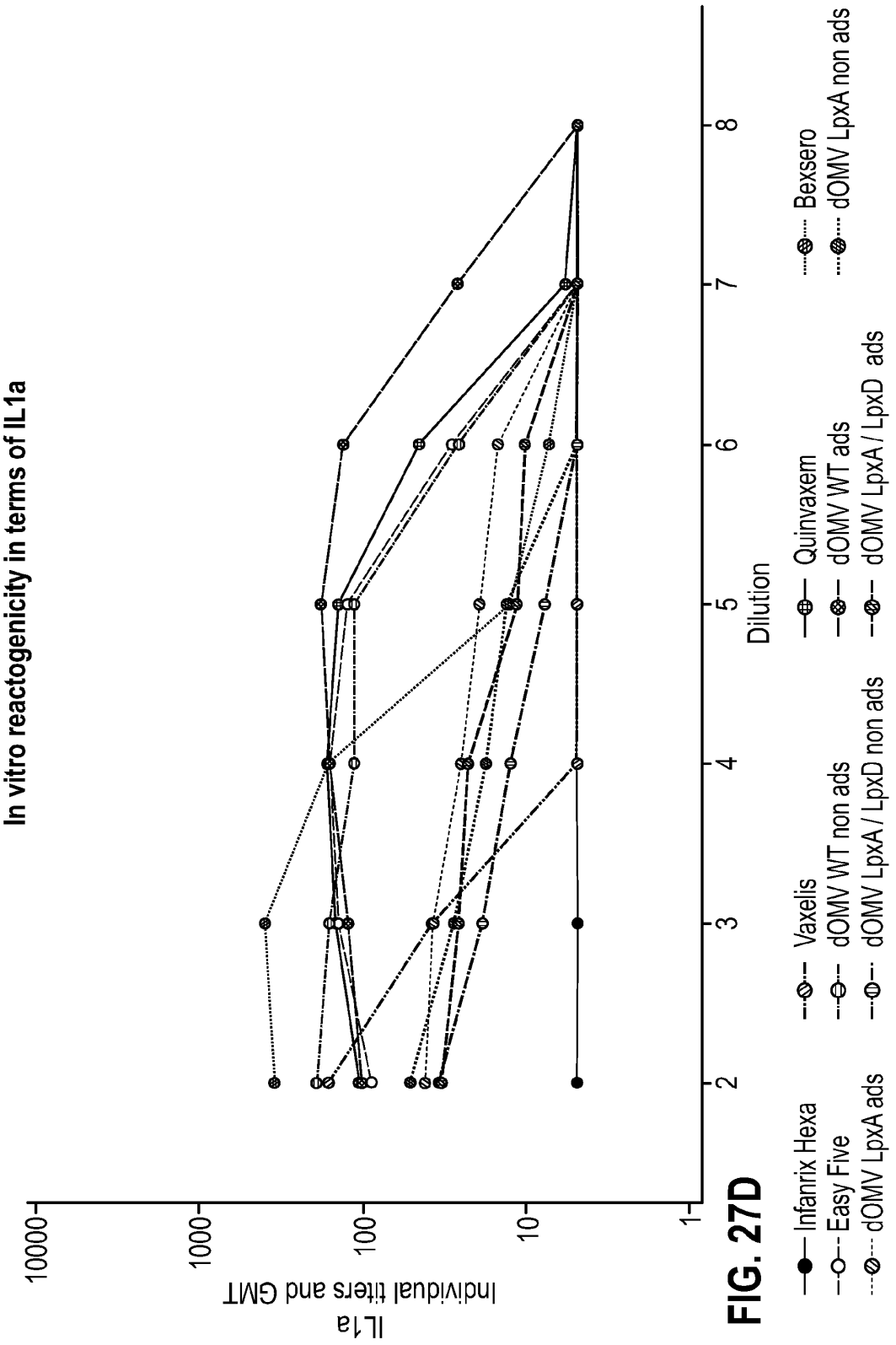

In vitro reactogenicity in terms of IL1a
IL1a
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Vaxelis
Quinvaxem
Bexsero
Easy Five
dOMV WT non ads
dOMV WT ads
dOMV LpxA non ads
dOMV LpxA ads
dOMV LpxA / LpxD non ads
dOMV LpxA / LpxD ads

FIG. 27D

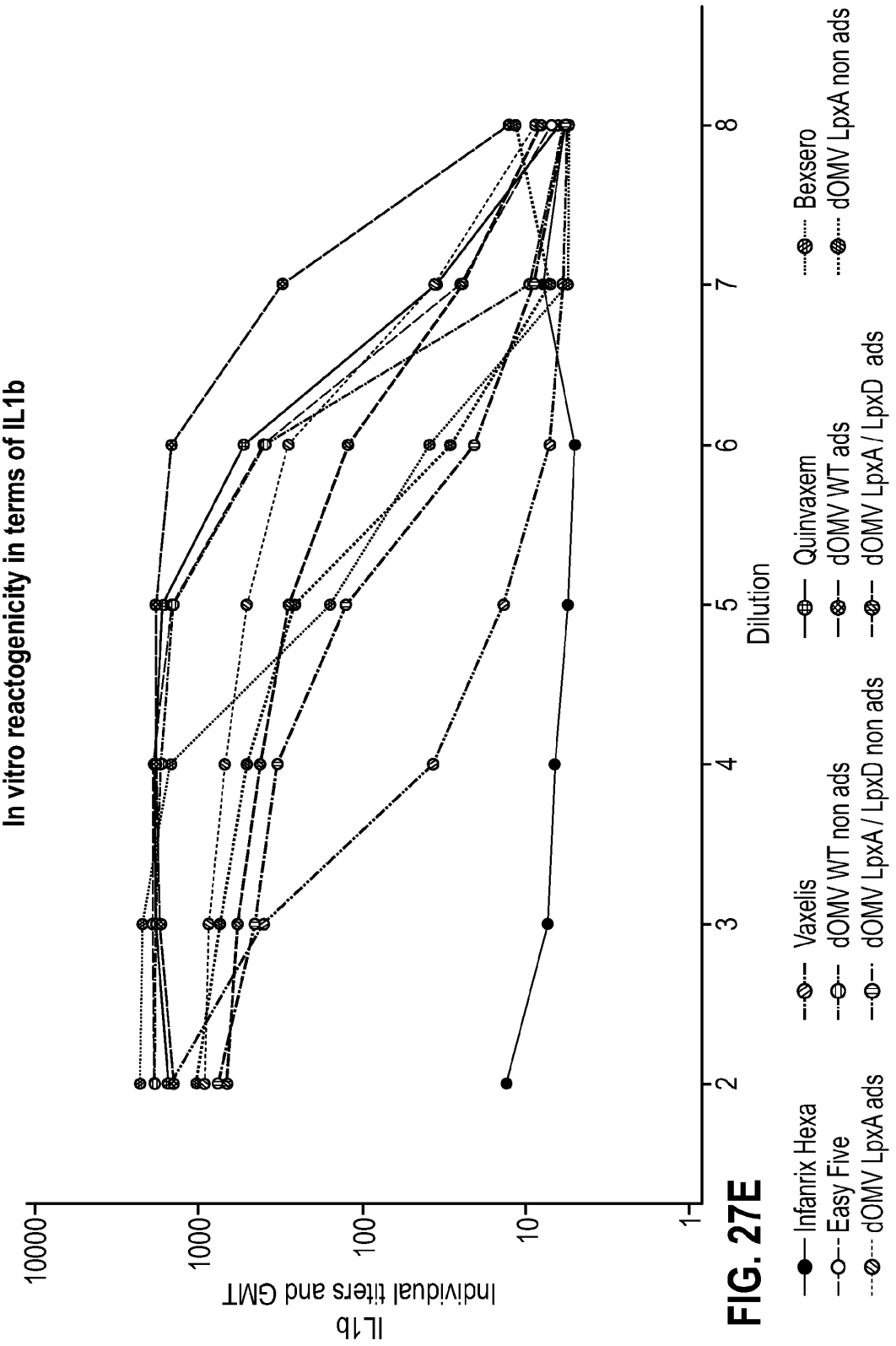

In vitro reactogenicity in terms of IL1b
IL1b
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Easy Five
dOMV LpxA ads
Vaxelis
dOMV WT non ads
dOMV LpxA / LpxD non ads
Quinvaxem
dOMV WT ads
dOMV LpxA / LpxD ads
Bexsero
dOMV LpxA non ads

FIG. 27E

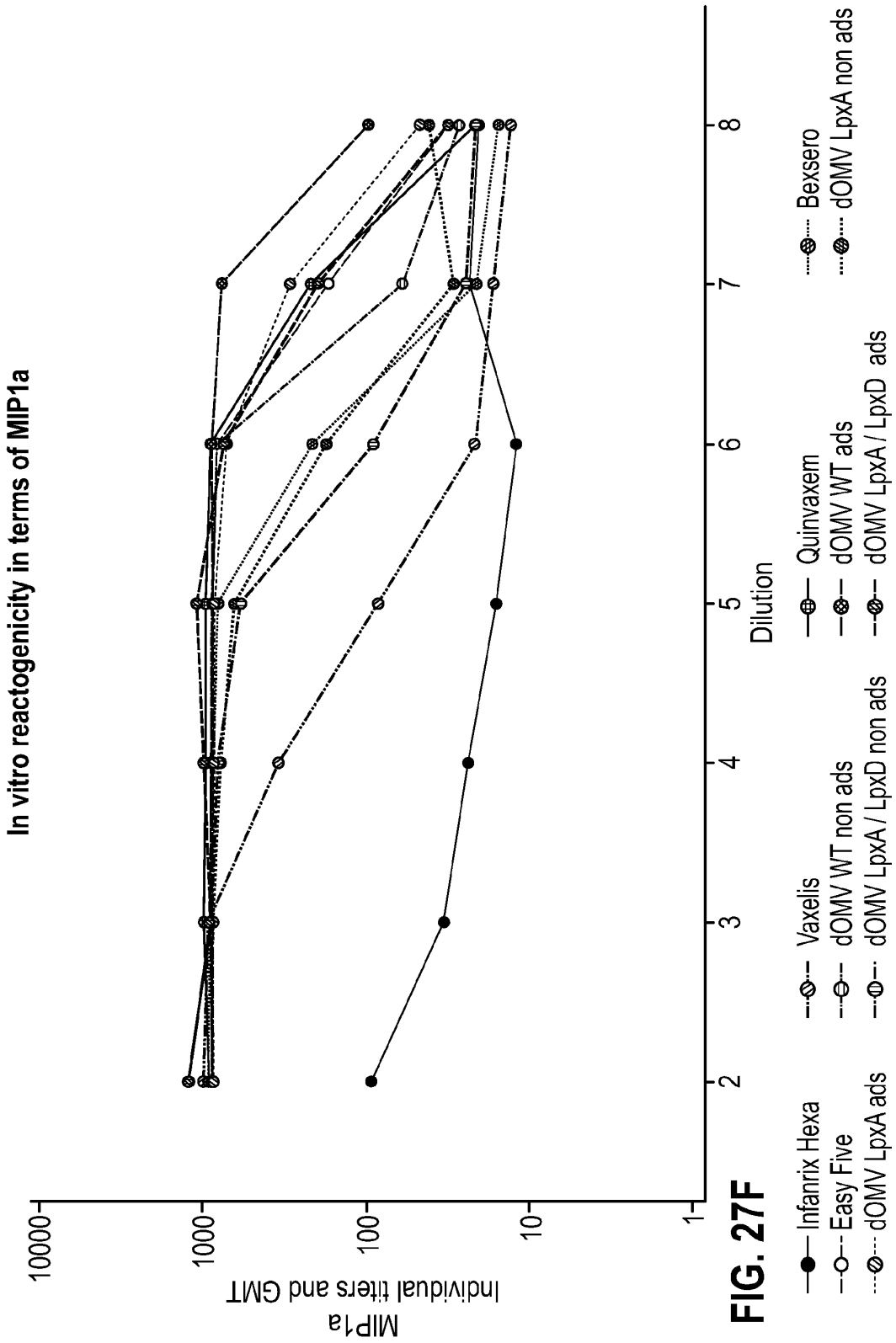

In vitro reactogenicity in terms of MIP1a
MIP1a
Individual titers and GMT
10000
1000
100
10
1
2
3
4
5
6
7
8
Dilution
Infanrix Hexa
Easy Five
dOMV LpxA ads
Vaxelis
dOMV WT non ads
dOMV LpxA / LpxD non ads
Quinvaxem
dOMV WT ads
dOMV LpxA / LpxD ads
Bexsero
dOMV LpxA non ads

FIG. 27F