

1 / 20

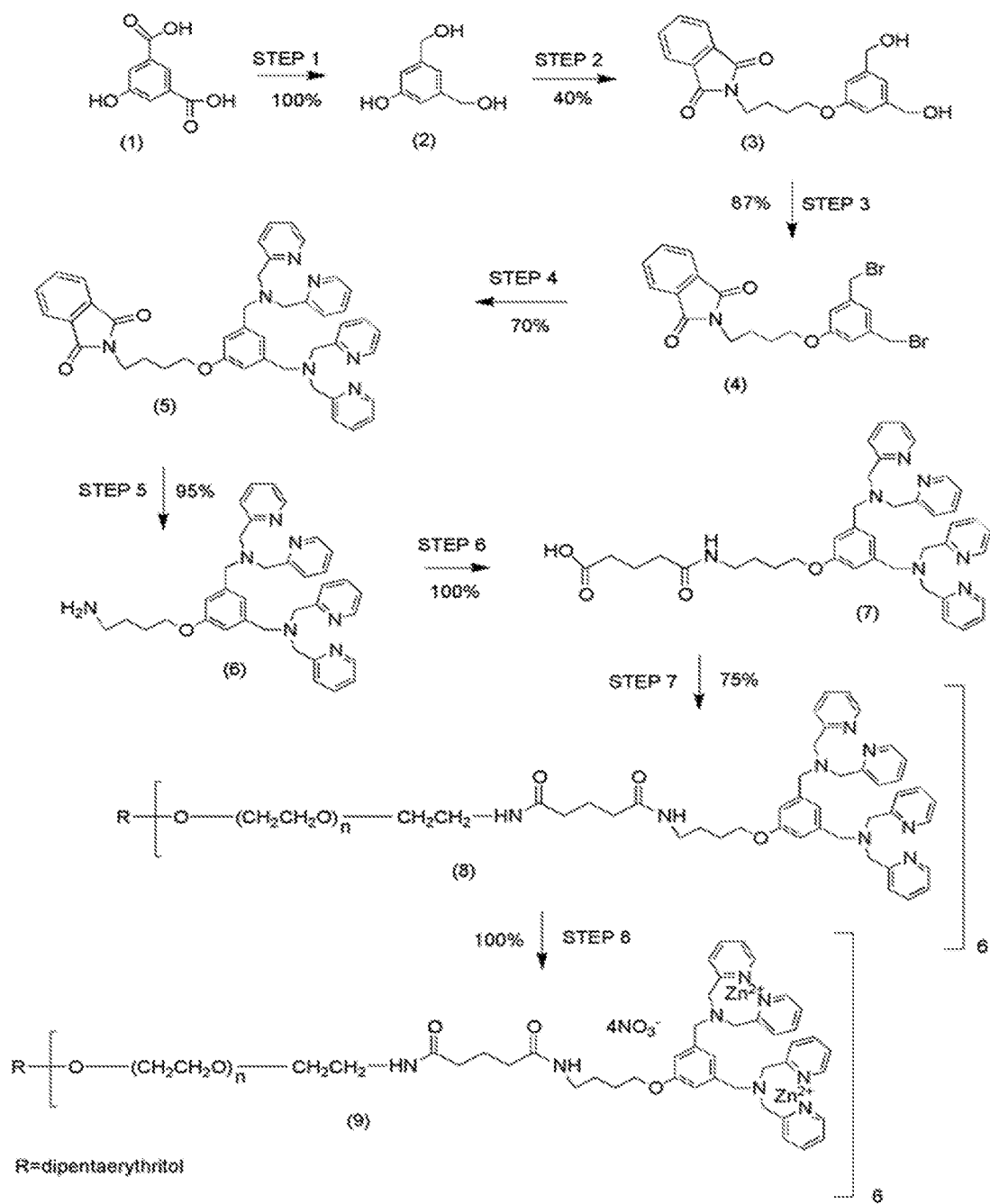


Figure 1

2 / 20

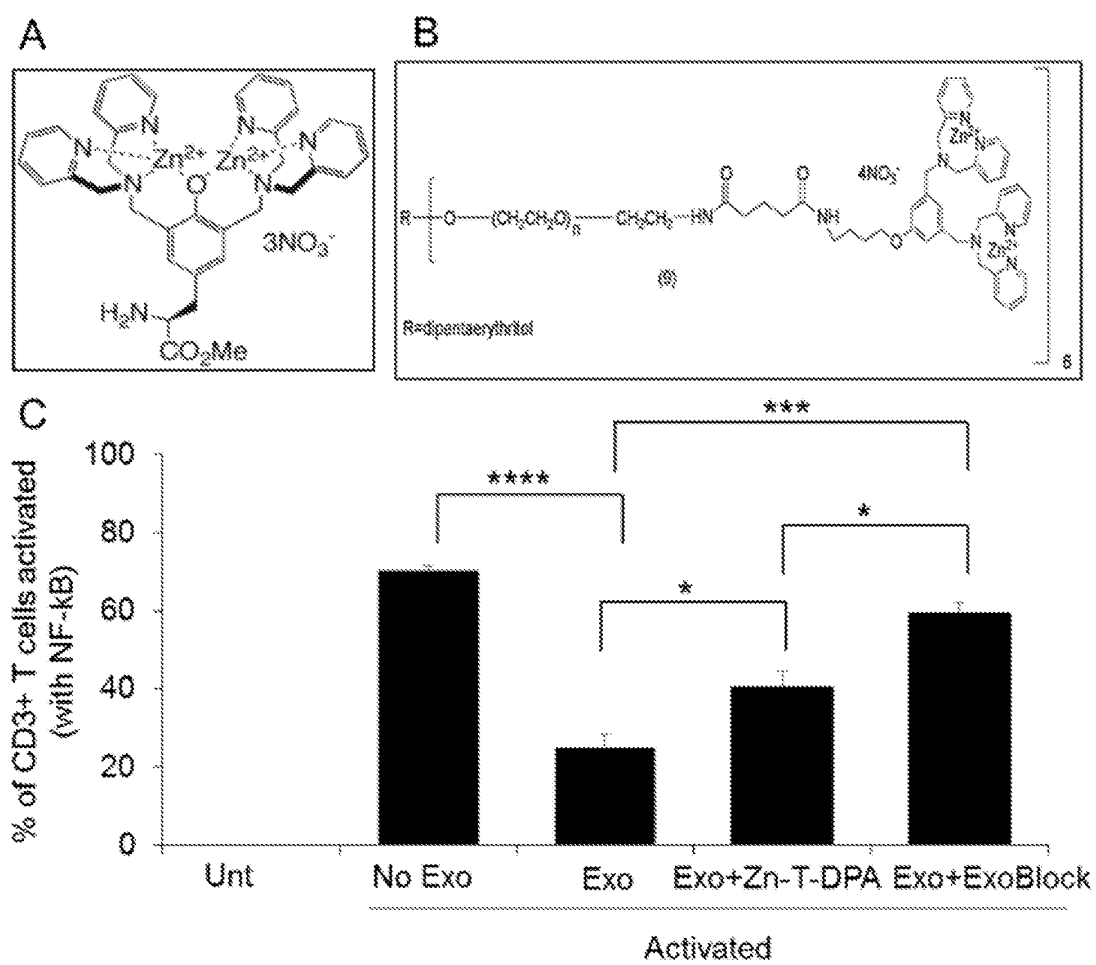


Figure 2

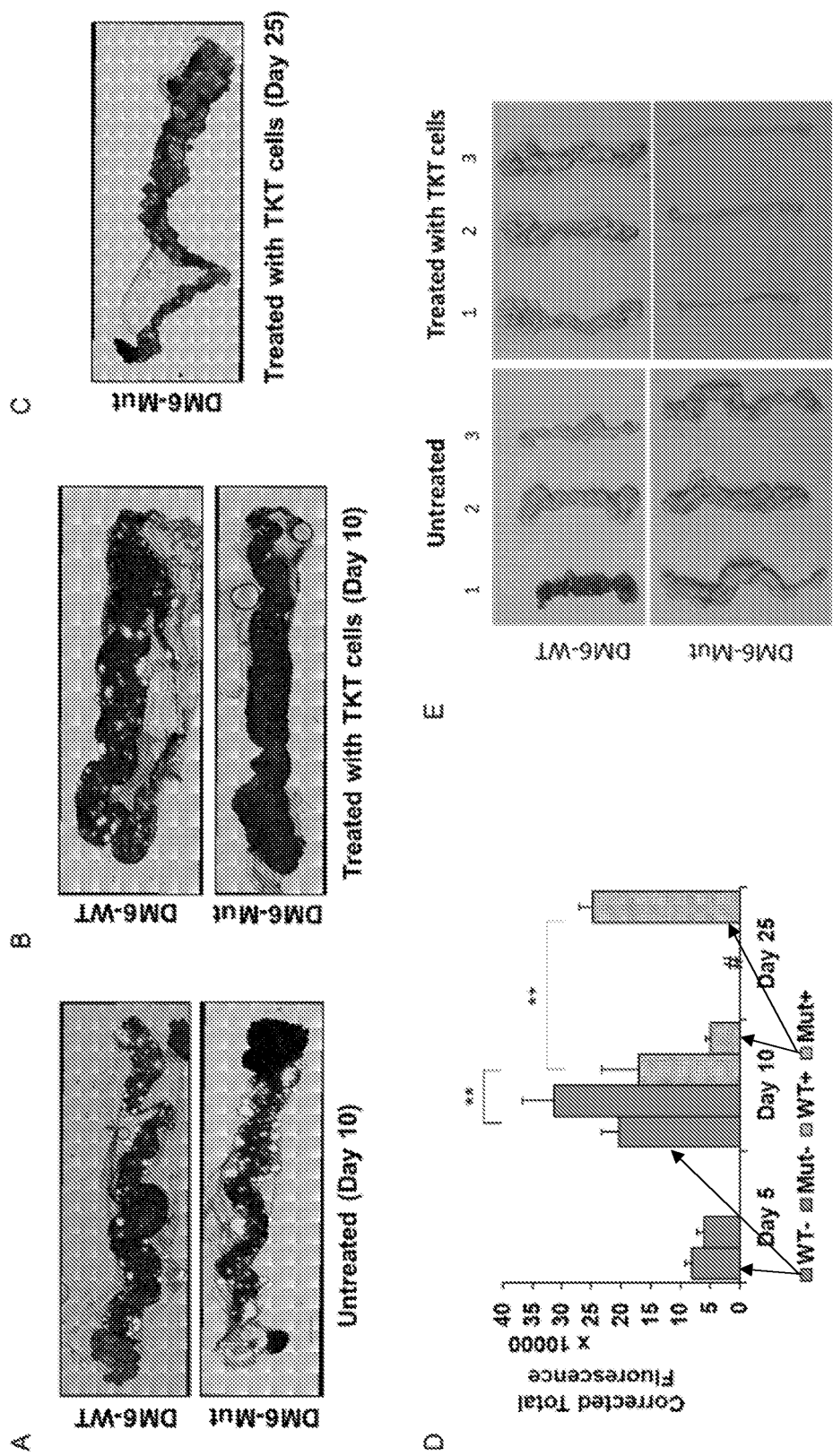


Figure 3

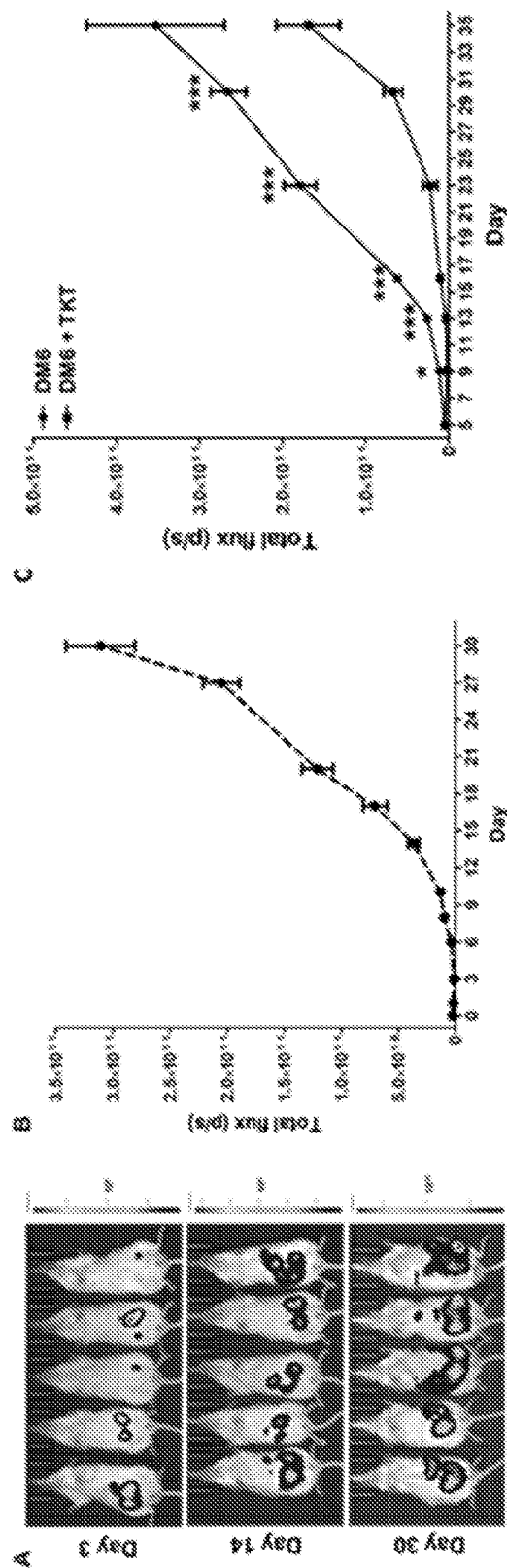


Figure 4

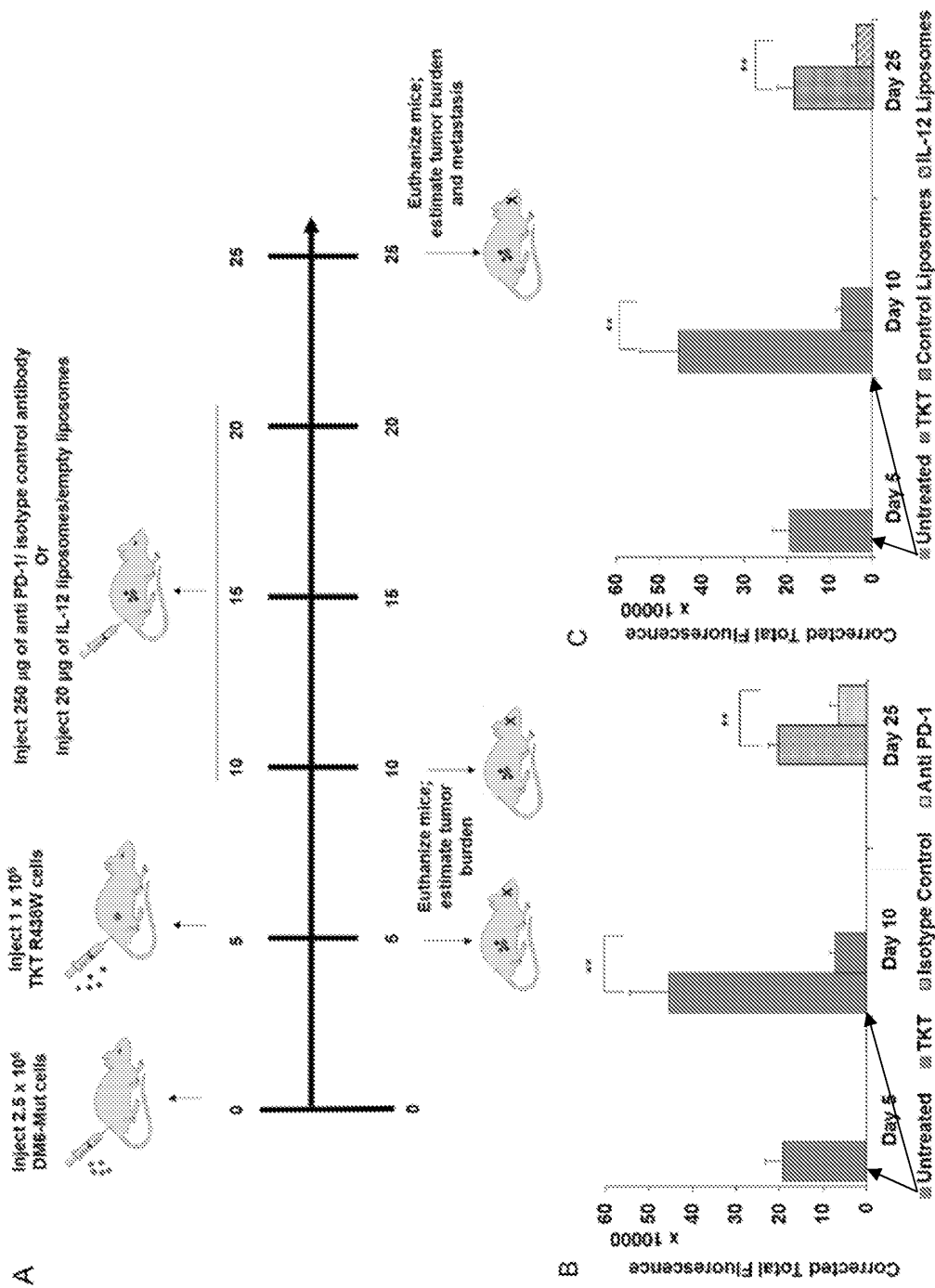


Figure 5

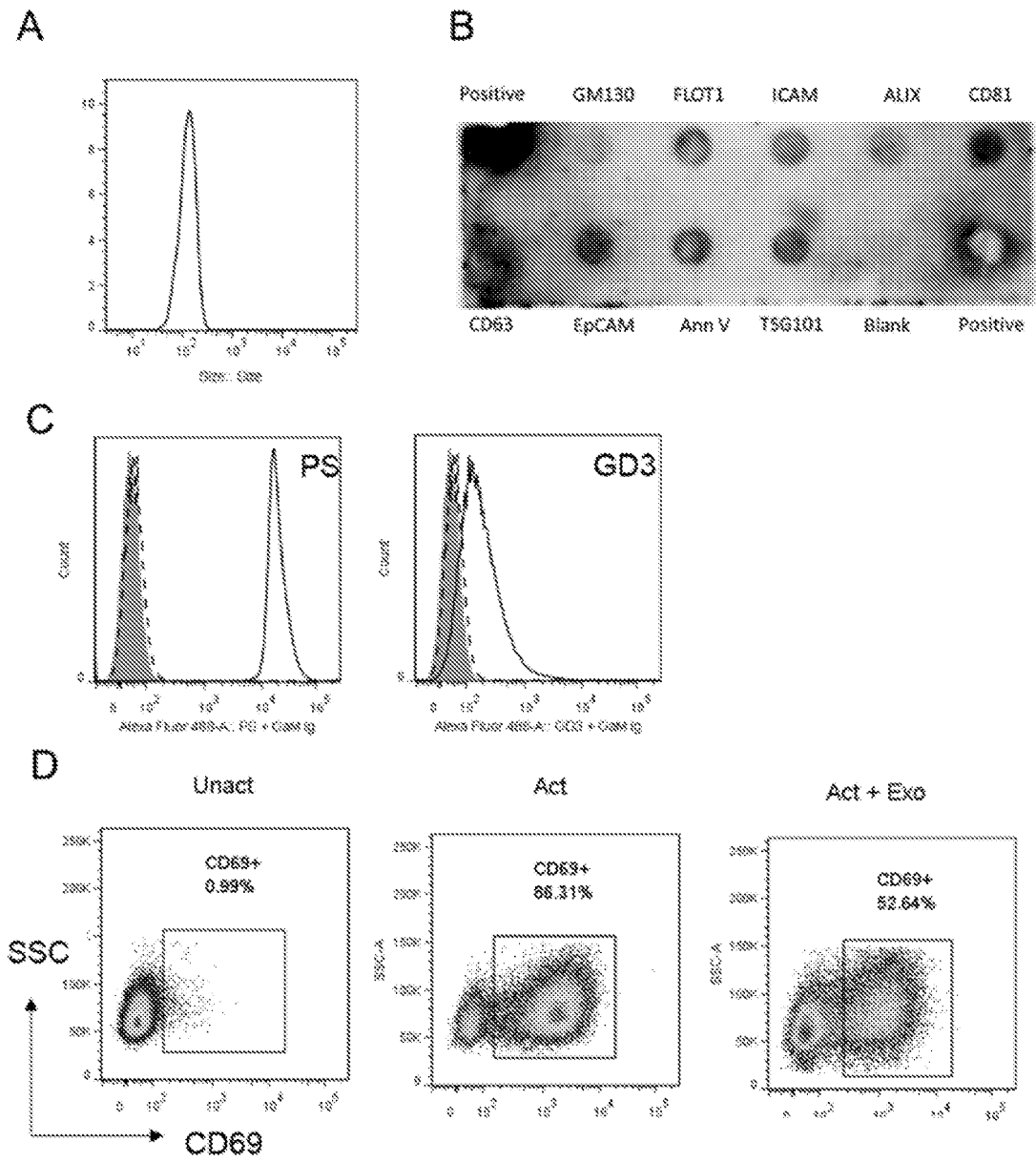


Figure 6

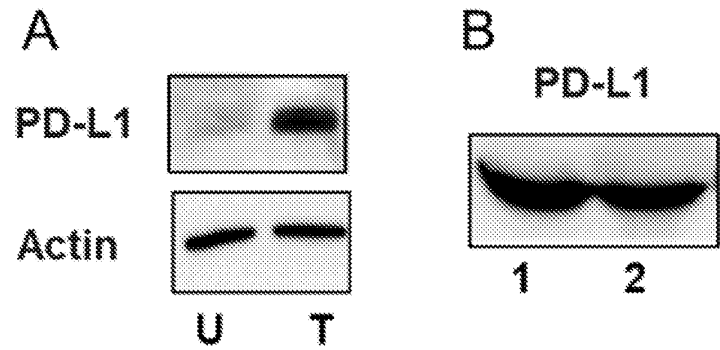


Figure 7

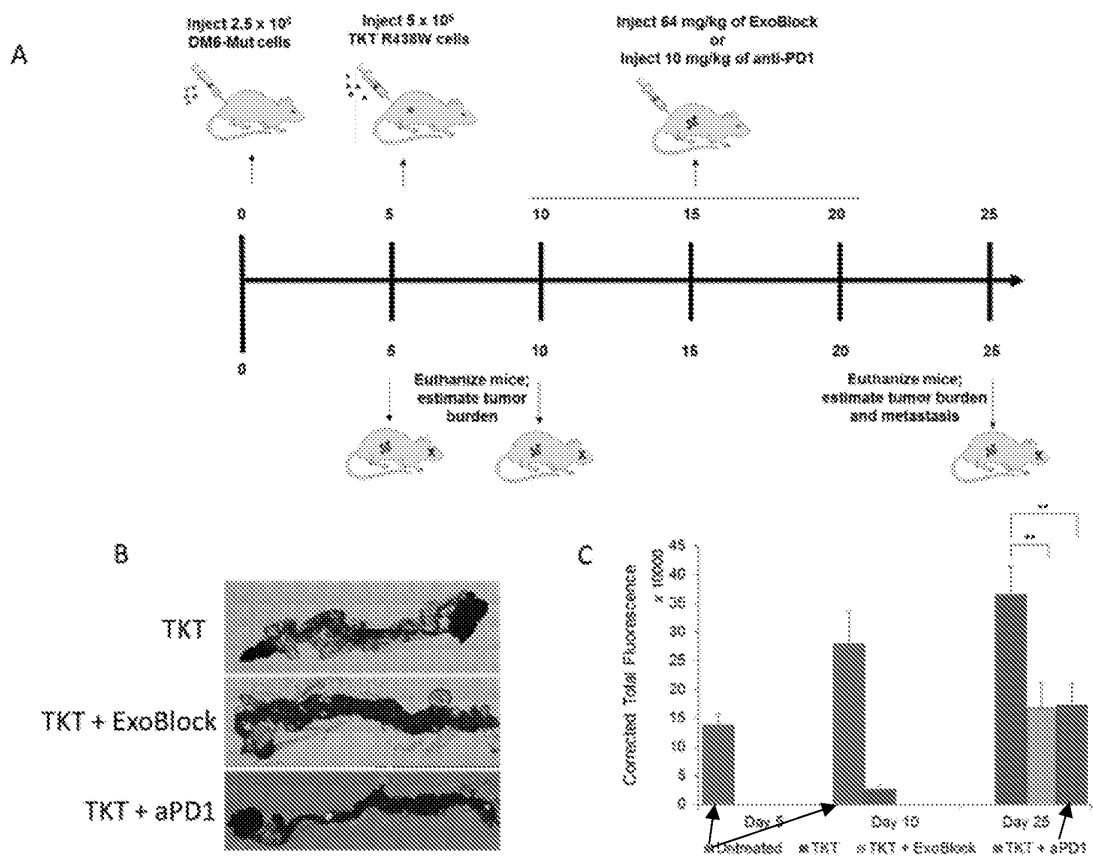


Figure 8

8 / 20

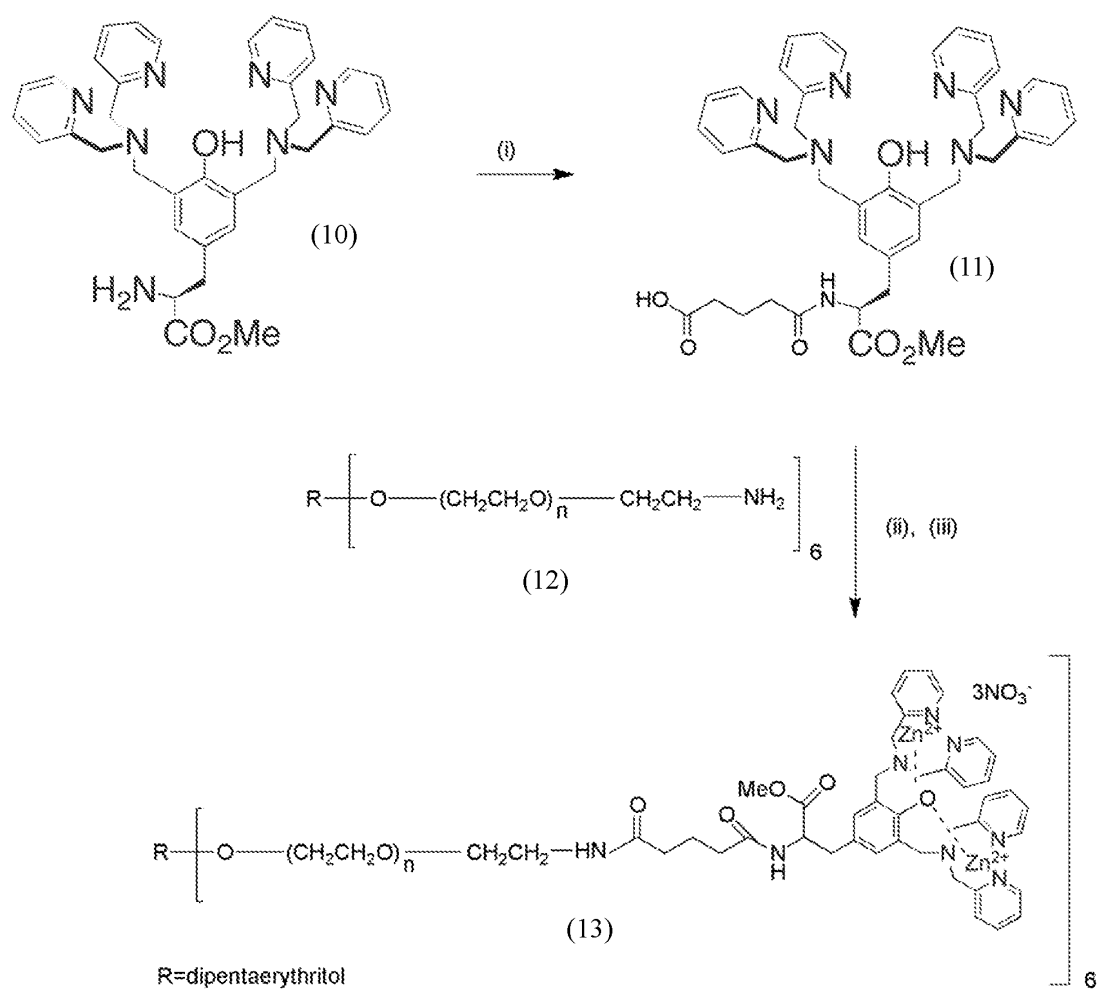


Figure 9

A

Set Up:

- One vial of ND-PBL 032615D was thawed & allowed to recover overnight in 1640+10% FCS.
- Cell count & viability:
At the time of thawing = 4.52×10^7 cells total, 97%V
After ON incubation in 1640 + 10%FCS = 2.89×10^7 cells total, 97%V
- On 12/5/16 Jenni depleted the exosomes from 5 – 4ml samples of 25% filtered RP091916 ovarian ascites fluid by ultracentrifugation at 200,000xg (45,900rpms) for 90 minutes at 4°C (with brake) in the Beckman L-90 Ultracentrifuge using the SW55Ti swing bucket rotor. The exosomes were brought up in 100% fresh 1640 + 1% HSA and stored overnight at 4°C.
- Exosomes in 100% 1640 + 1% HSA were treated with 3, 10 or 30μM (Zn-DPA)6-PEG for 1 hour at 37°C to allow the (Zn-DPA)6-PEG to bind to phosphatidylserine on the exosomes. The exosomes were then brought up to 1ml with 1640 + 1% HSA (1X final concentration).
Gift from Molecular Targeting Technologies, MW = 21,366, Lot #mtti-039-032
1mM stock of (Zn-DPA)6-PEG in 1640 0% prepared 11/28/16 and stored at 4°C
- The Nunc Maxisorp tubes were coated for activation with 0.1μg OKT3 + 5μg αhuCD28 in PBS in 500% total volume. They were coated overnight at 4°C.
- The activation was done in 1640 + 1% HSA with or without 3, 10 or 30μM (Zn-DPA)6-PEG or 1X exosomes in 1640 + 1% HSA with or without 3, 10 or 30μM (Zn-DPA)6-PEG for 2 hours at 37°C, 5×10^5 cells/tube. The cells were then stained as usual for intracellular CD3 and NFκB.

Figure 10

B

Activation Media	Condition	% of CD3+ Cells with Nuclear NFkB	% Inhibition of CD3+ Cells with Nuclear NFkB
1640 + 1% HSA	Unactivated	0 (0/100)	-
1640 + 1% HSA	PMA + Ionomycin	96 (96/100)	-
1640 + 1% HSA	α -huCD3/CD28	67 (268/400)	-
1640 + 1% HSA + 3 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	59 (235/400)	-
1640 + 1% HSA + 10 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	59 (234/400)	-
1640 + 1% HSA + 30 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	72 (286/400)	-
1640 + 1% HSA + 30 μ M (Zn-DPA)6-PEG	PMA + Ionomycin	98 (98/100)	-
1X Exosomes	α -huCD3/CD28	29 (117/400)	57
1X Exosomes + 3 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	58 (232/400)	13
1X Exosomes + 10 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	65 (259/400)	3
1X Exosomes + 30 μ M (Zn-DPA)6-PEG	α -huCD3/CD28	64 (255/400)	4
1X Exosomes + 30 μ M (Zn-DPA)6-PEG	PMA + Ionomycin	100 (100/100)	-

ND-PBL 032615D & Exosomes from RP091916 Ovarian Ascites Fluid were used in this experiment.
A 1mM Zn-T-DPA stock was prepared in 1640 0% 11/28/16 and stored at 4°C (Gift from Molecular Targeting Technologies)
Activation time = 2 hours at 37°C

Conclusions:

- 1) Exosomes from RP091916 Ovarian Ascites Fluid inhibited the translocation of NFkB by 57%
- 2) There was a 77% reversal of the inhibition when ND-PBL activated in the presence of exosomes + 3 μ M (Zn-DPA)6-PEG
- 3) There was a 95% reversal of the inhibition when ND-PBL activated in the presence of exosomes + 10 μ M (Zn-DPA)6-PEG
- 4) There was a 93% reversal of the inhibition when ND-PBL activated in the presence of exosomes + 30 μ M (Zn-DPA)6-PEG

Figure 10 (cont.)

11 / 20

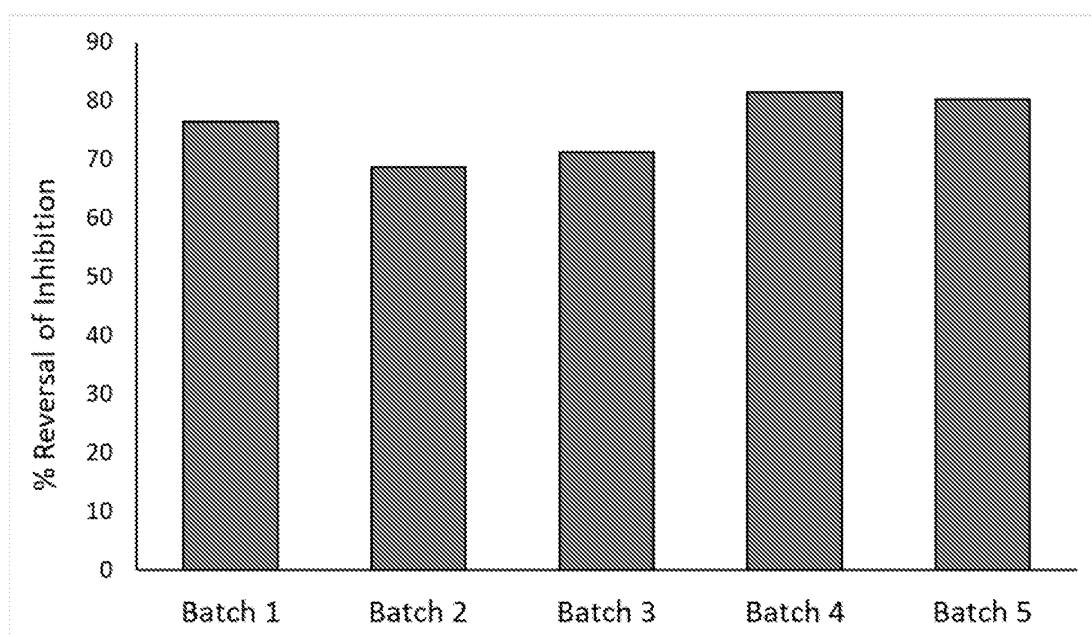


Figure 11

Apoptotic Cells

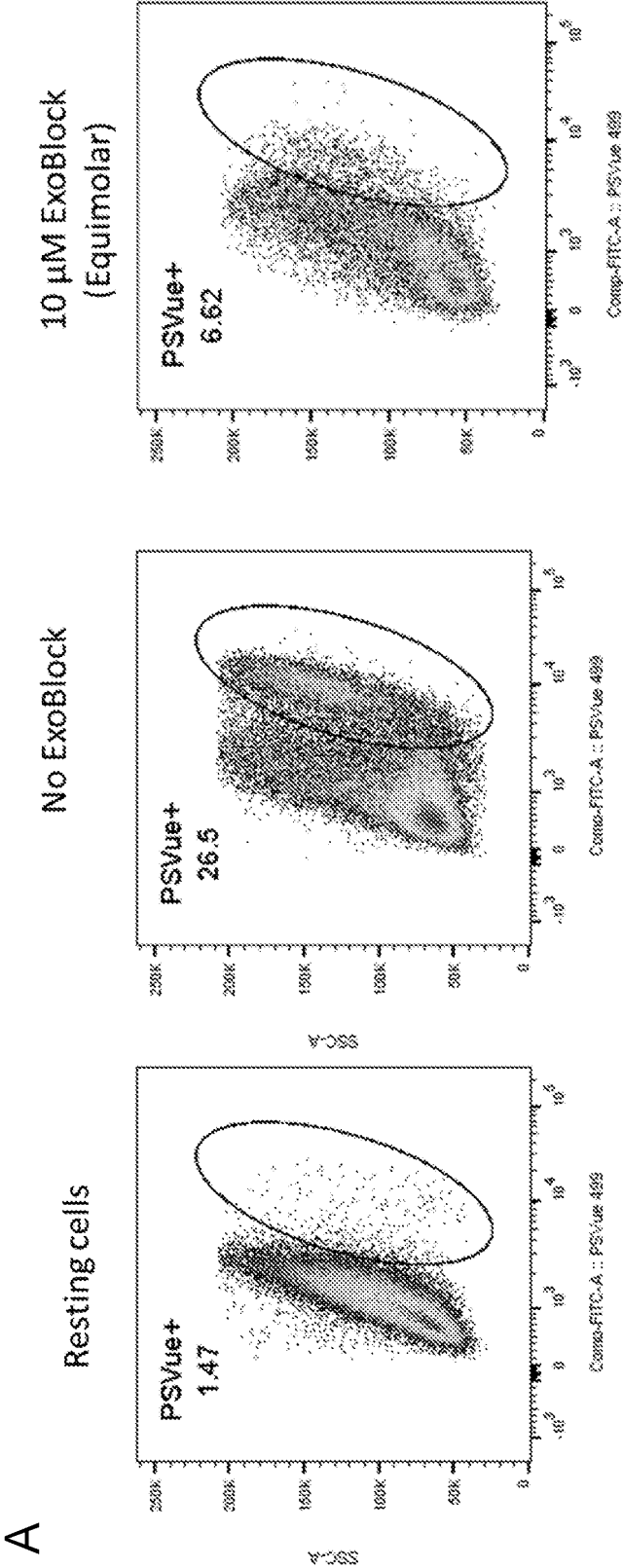


Figure 12

13 / 20

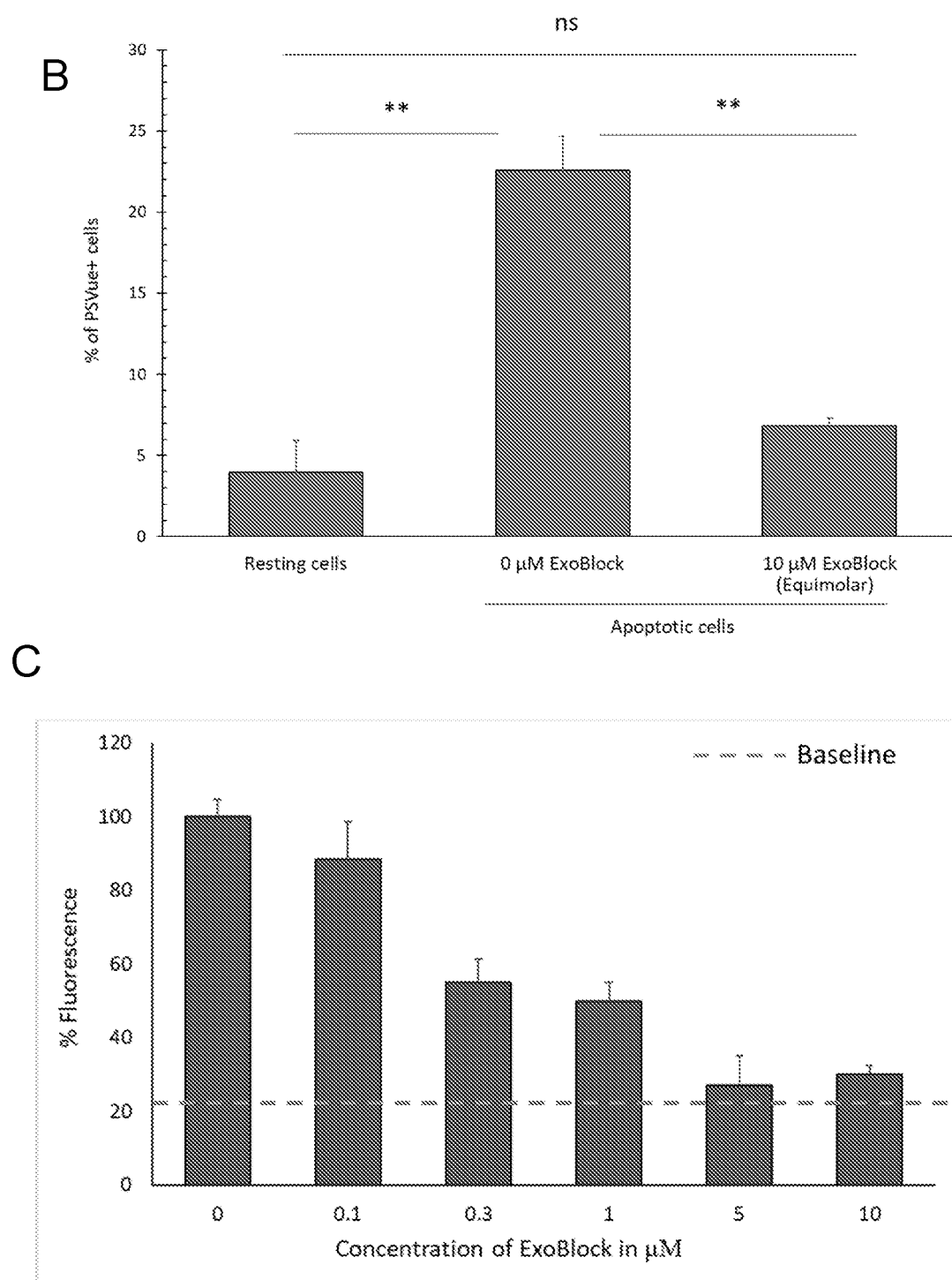


Figure 12 (cont.)

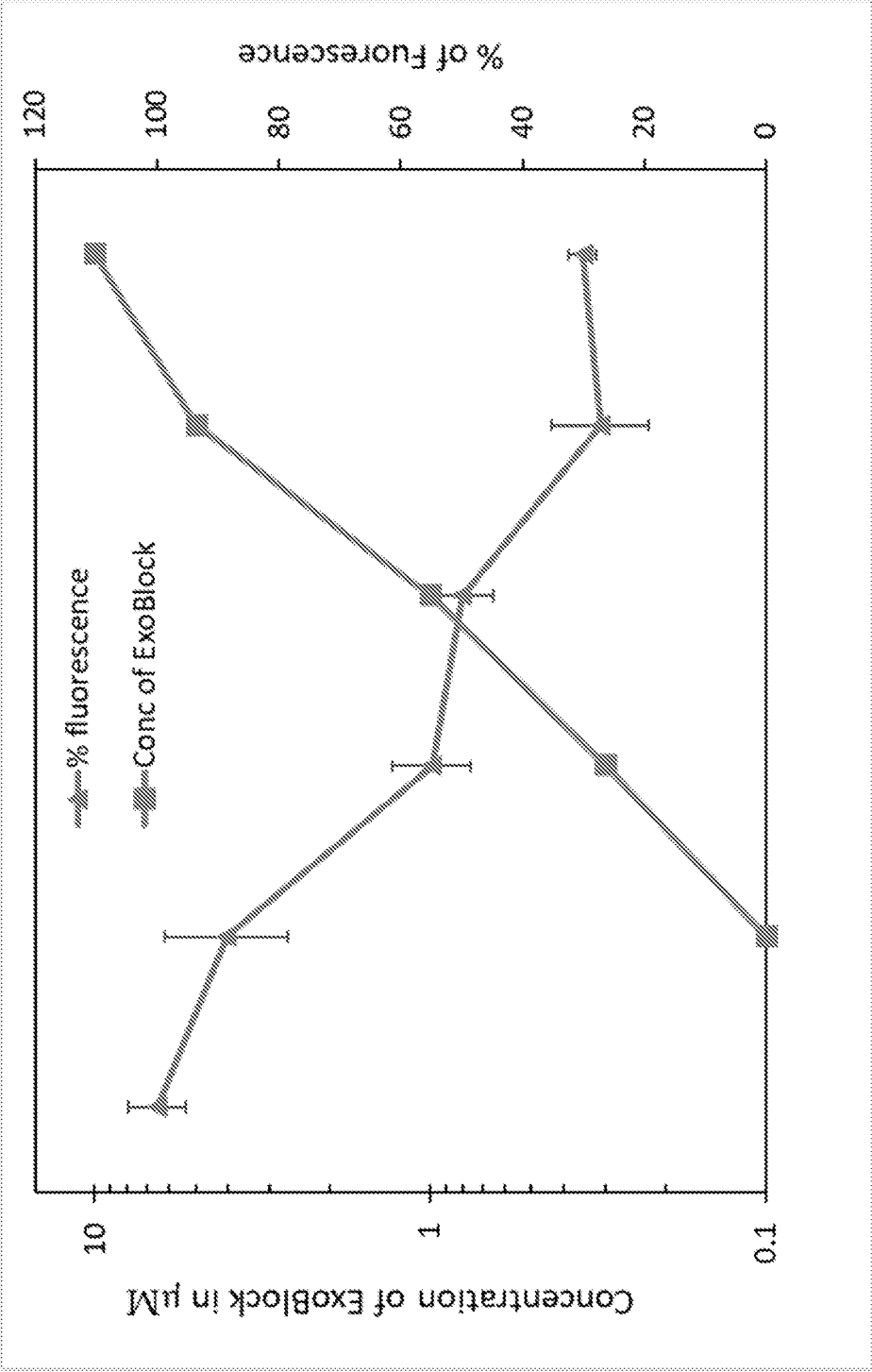


Figure 12 (cont.)

D

15 / 20

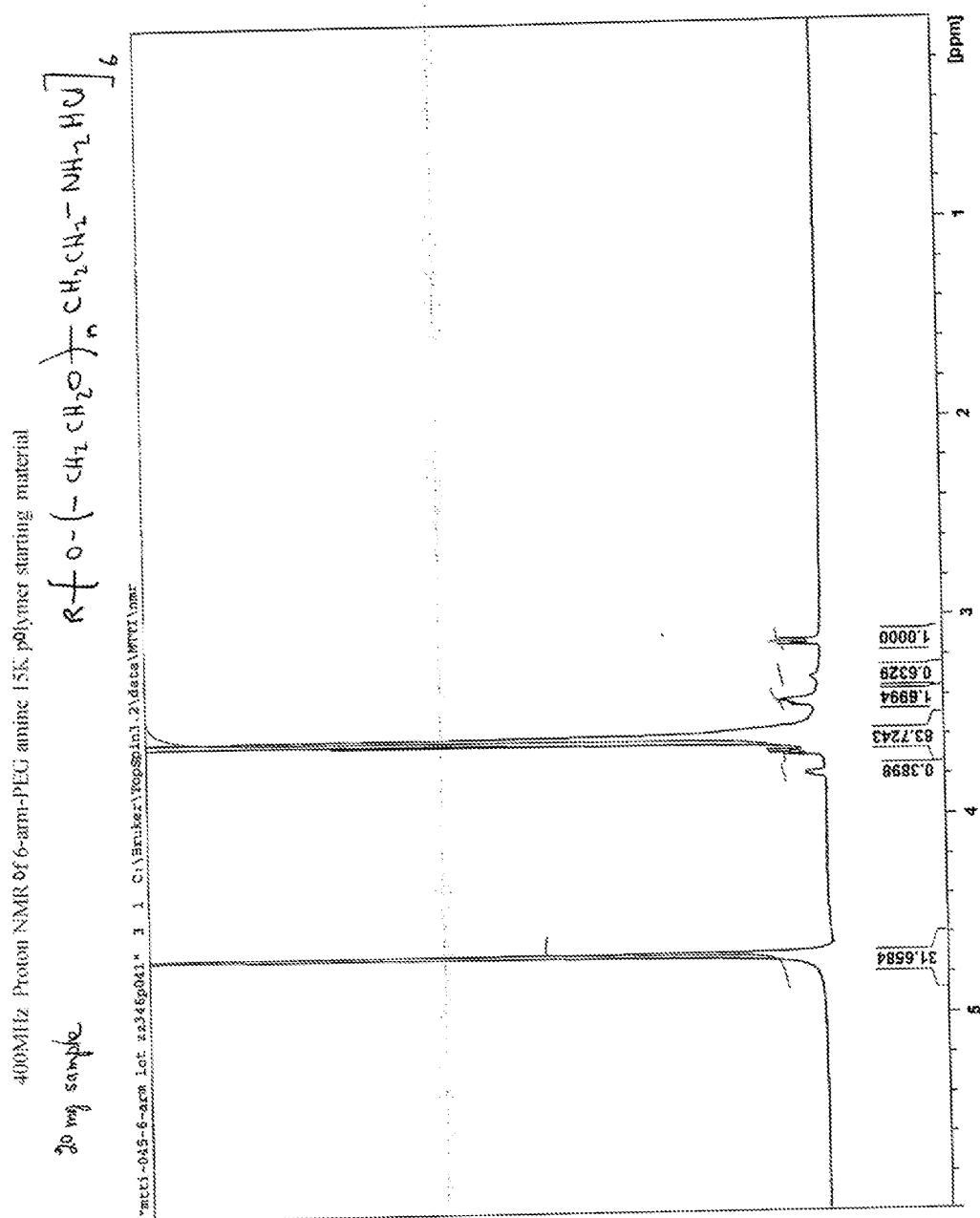


Figure 13

A

400 MHz Proton NMR of ExoBlock batch # mfg-045-174-1

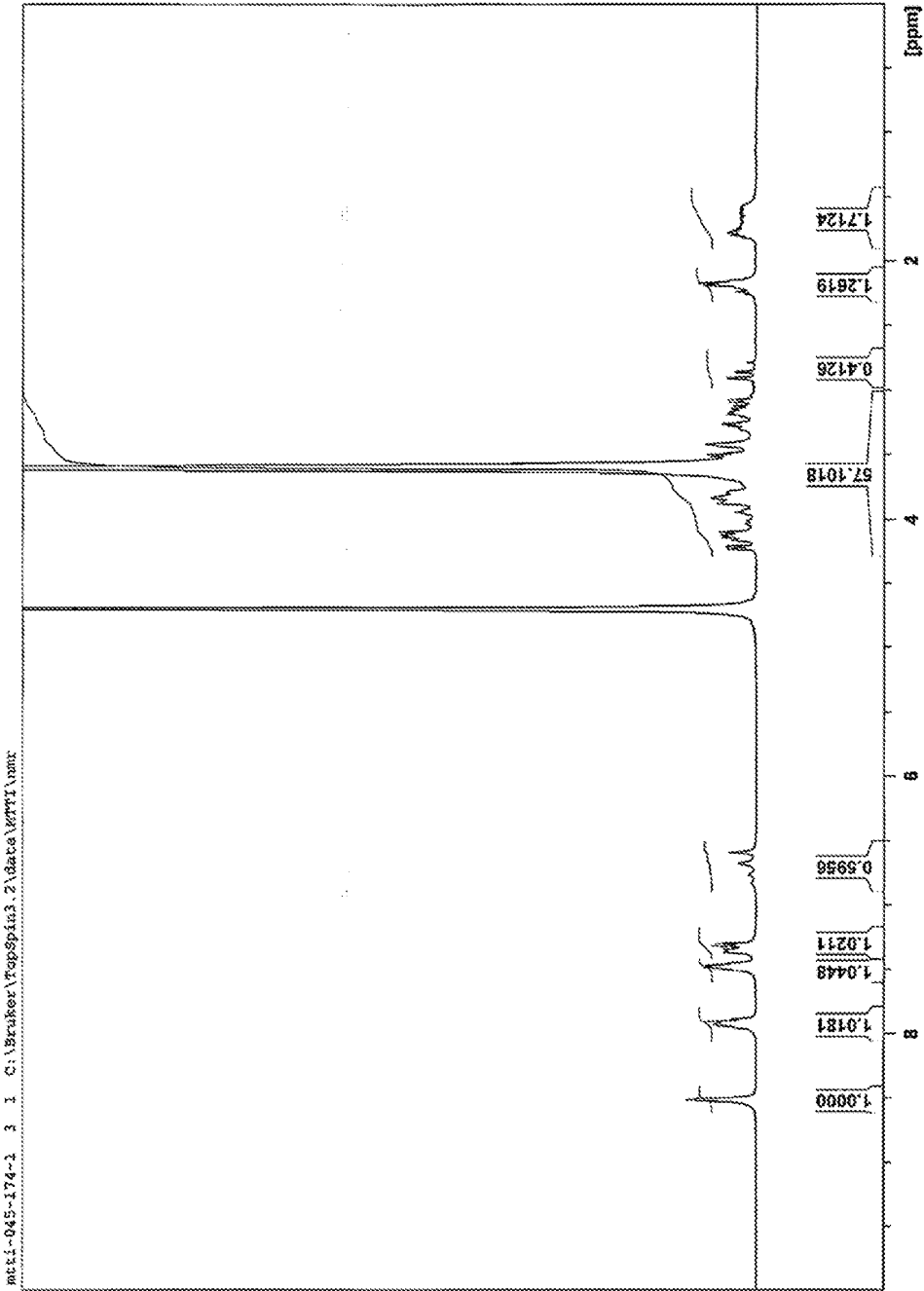


Figure 13 (cont.)

B

400 MHz Proton NMR of ExoBlock batch # mti-045-181

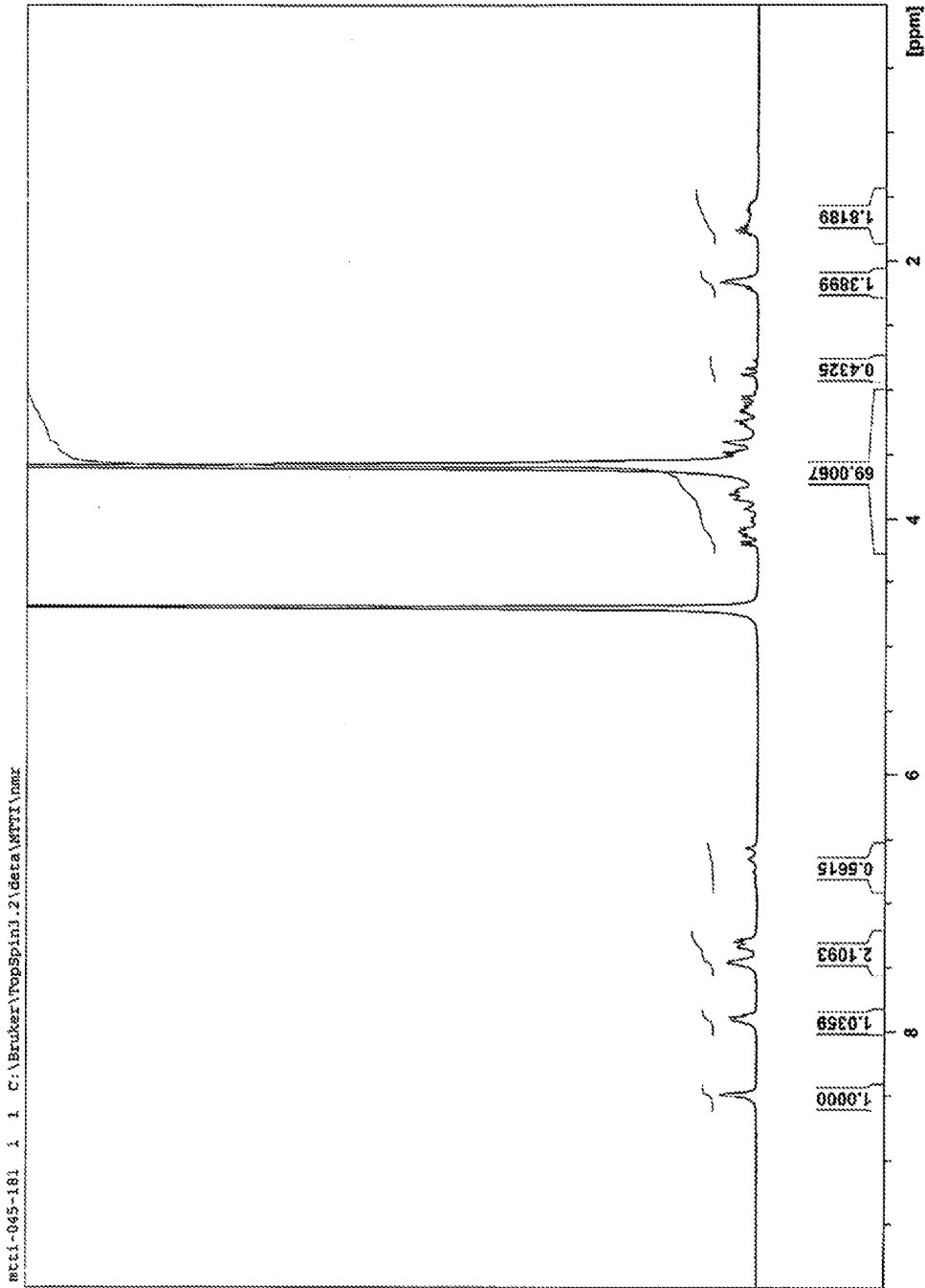


Figure 13 (cont.)

C

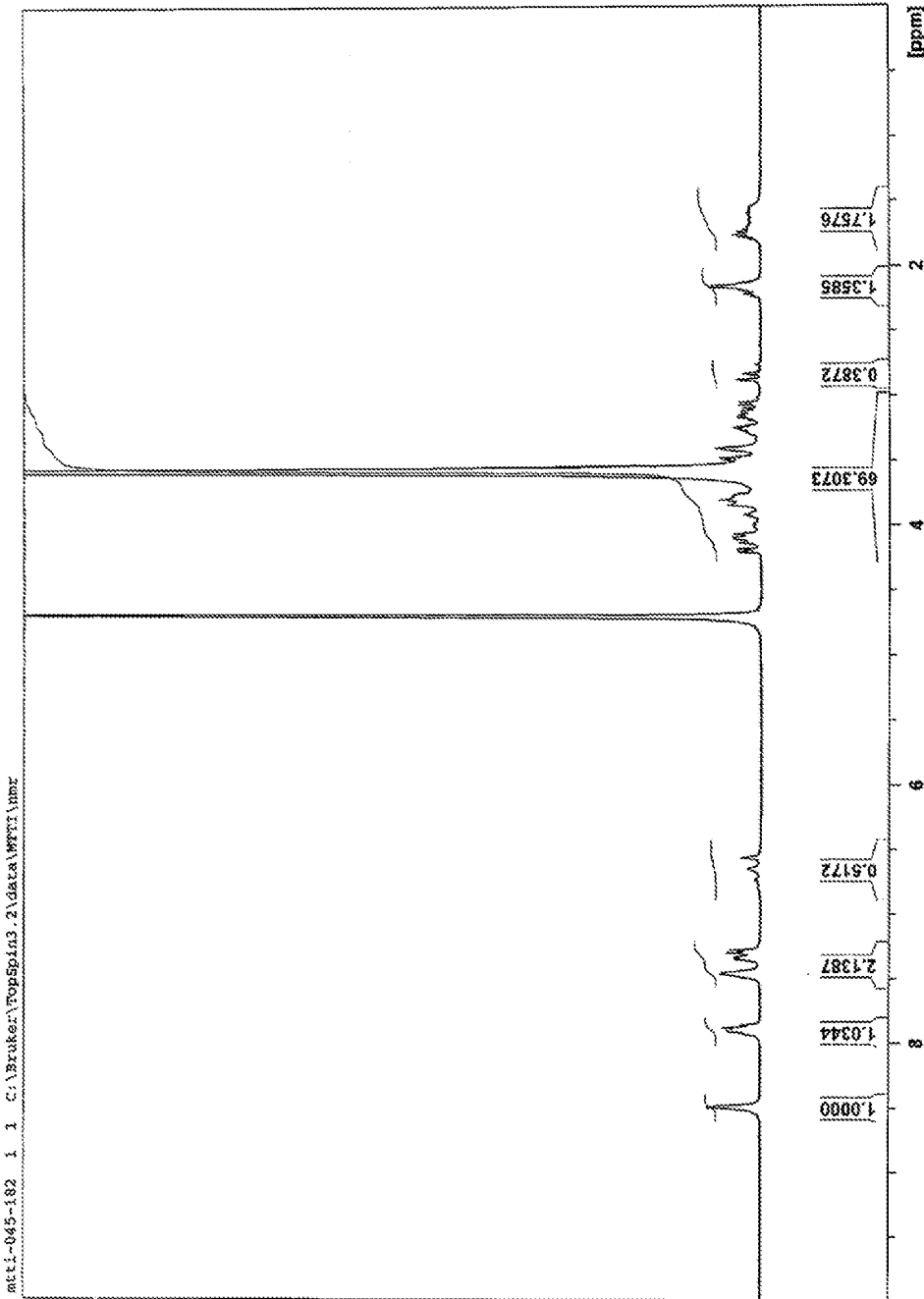


Figure 13 (cont.)

D

400MHz PROTON NMR OF EXOBLOCK BATCH # MTTL-045-186

mttl-045-186 2 1 C:\arker\gcp\pin3.2\data\MTTL\mmr

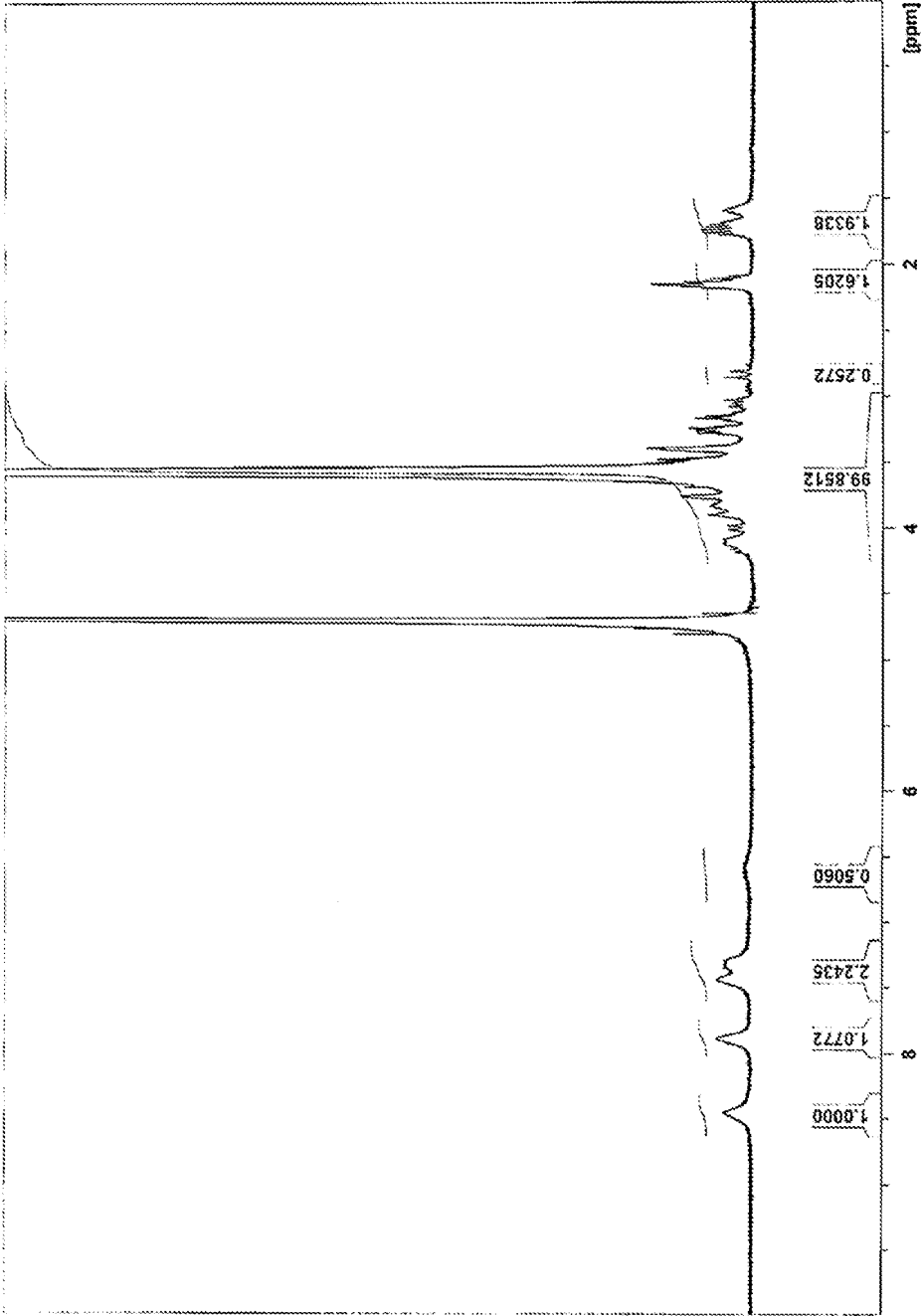


Figure 13 (cont.)

E

400MHz PROTON NMR OF EXOBLOCK BATCH # MITT-045-187

mitt-045-187 2 1 C:\Bruker\TopSpin3.2\data\mitt\

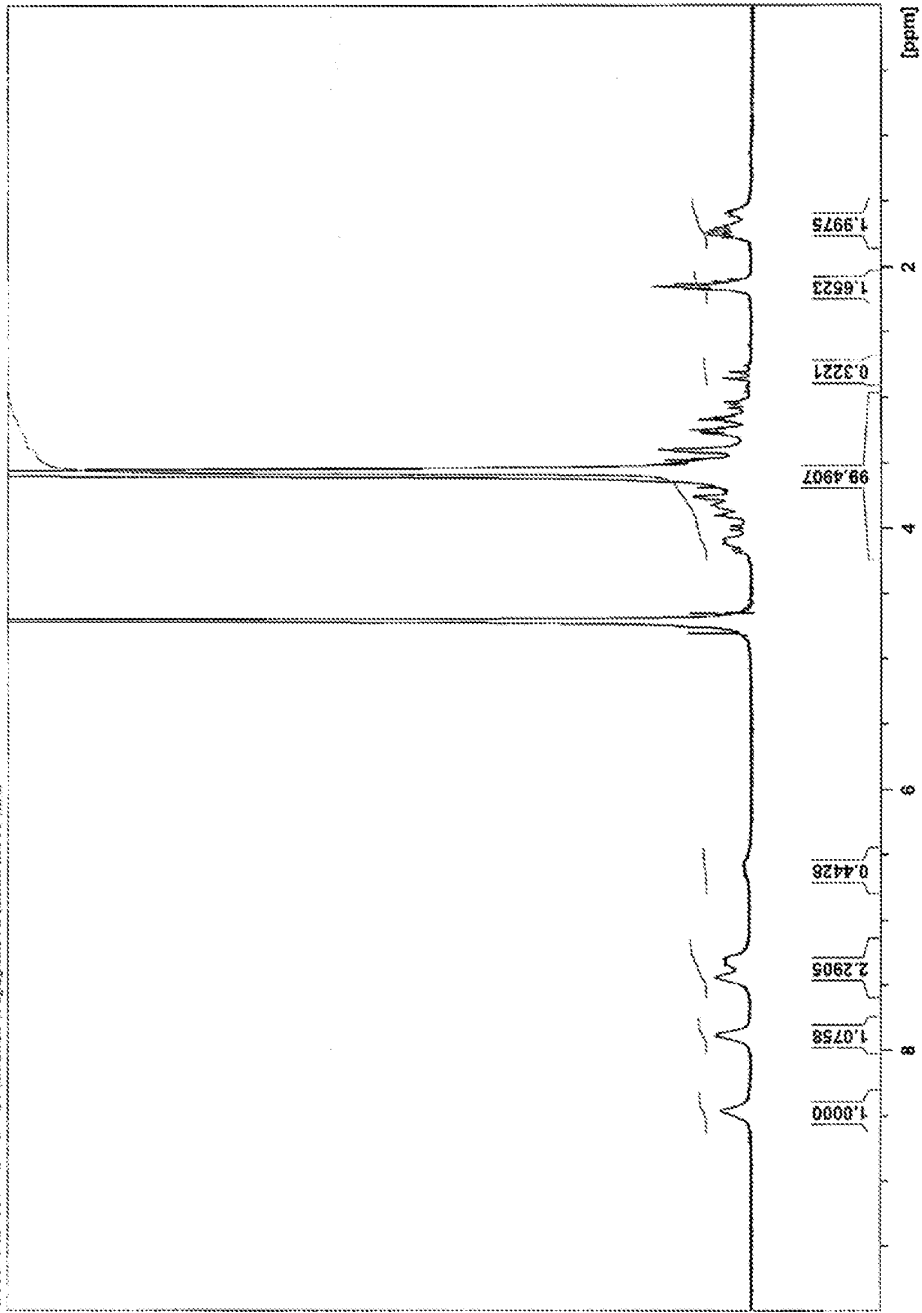


Figure 13 (cont.)