2-Cl-Trt-Resin + 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid

↓ DIPEA

2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-(2-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid, DIPC +HOBt

2-[2-[2-[[2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-[2-[[2-[2-(2-aminoethoxy)ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ Fmoc-Glu-OtBu +DIPC+HOBt

2-[2-[2-[[2-[2-[2-[[(4*S*)-4-Fmoc-amino-5-tert-butoxy-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy] ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-[2-[[2-[2-[2-[[(4*S*)-4-amino-5-tert-butoxy-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy] acetic acid-2-Cl-Trt-Resin

↓ Octadecanedioic acid mono tert-butyl ester +DIPC+HOBt

[2-[2-[2-[[2-[2-[2-[[(4*S*)-5-tert-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid]- 2-Cl-Trt-Resin

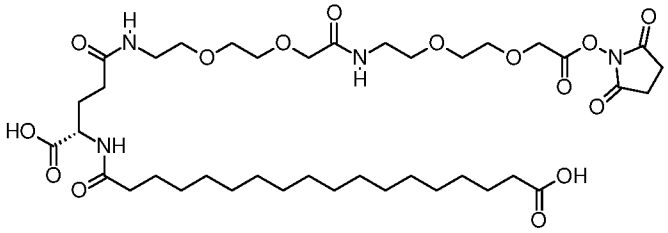
↓ Trifluoroethanol:DCM (1:1)

2-[2-[2-[[2-[2-[2-[[(4*S*)-5-tert-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

↓ IBCF+NMM+HOSu

tert-butyl 18-[[(1*S*)-1-*tert*-butoxycarbonyl-4-[2-[2-[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoate

↓ TFA



**Moiety A-OSu**

**Figure 1A**

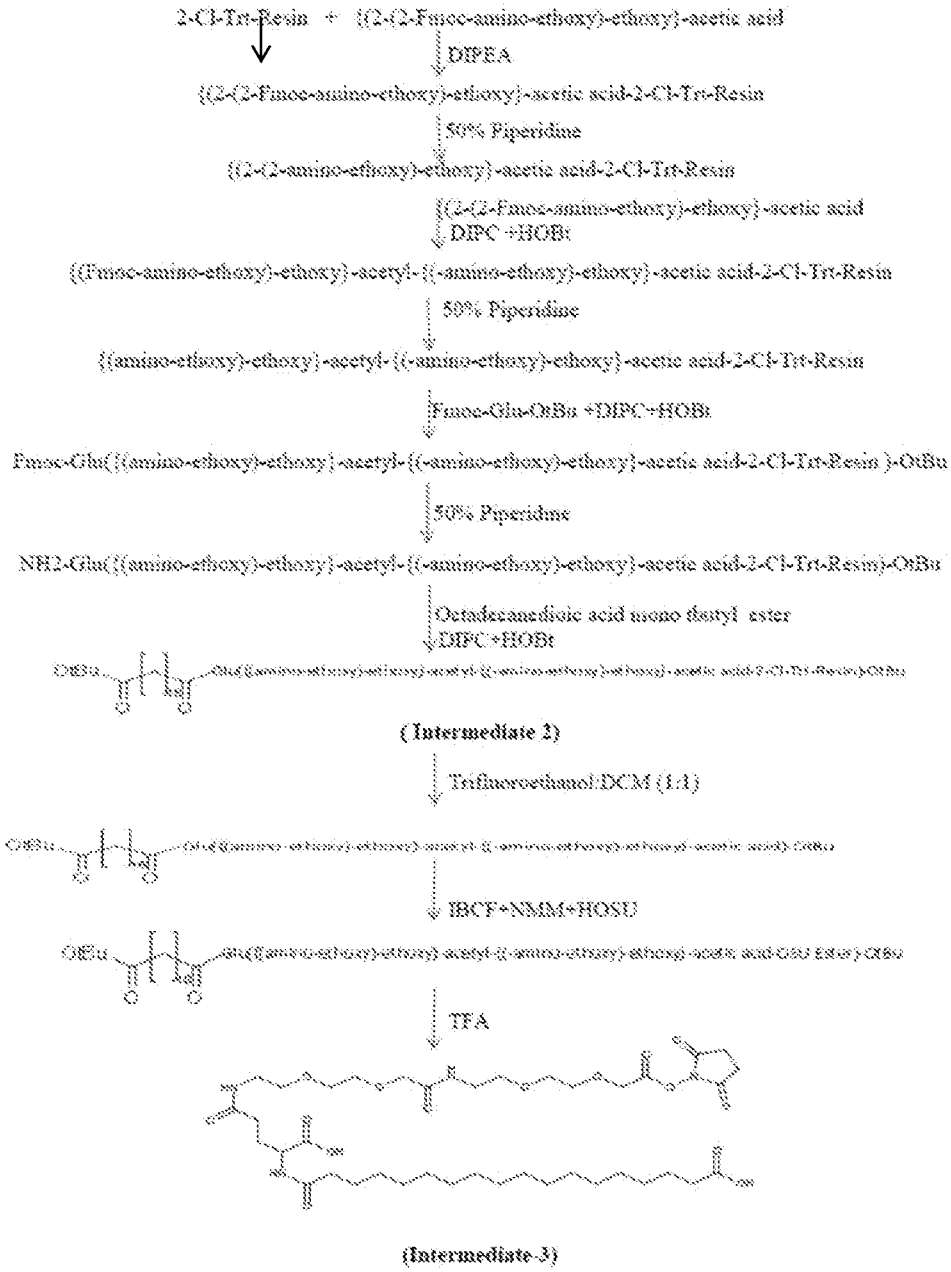

2-Cl-Trt-Resin + {(2-(2-Fmoc-amino-ethoxy)-ethoxy}-acetic acid
DIPEA
{(2-(2-Fmoc-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin
50% Piperidine
{(2-(2-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin
{(2-(2-Fmoc-amino-ethoxy}-ethoxy}-acetic acid
DIPC +HOBt
{(Fmoc-amino-ethoxy}-ethoxy}-acetyl-{(-amino-ethoxy}-ethoxy}-acetic acid-2-Cl-Trt-Resin
50% Piperidine
{(amino-ethoxy}-ethoxy}-acetyl-{(-amino-ethoxy}-ethoxy}-acetic acid-2-Cl-Trt-Resin
Fmoc-Glu-OtBu +DIPC+HOBt
Fmoc-Glu({(amino-ethoxy}-ethoxy}-acetyl-{(-amino-ethoxy}-ethoxy}-acetic acid-2-Cl-Trt-Resin }-OtBu
50% Piperidine
NH2-Glu({(amino-ethoxy}-ethoxy}-acetyl-{(-amino-ethoxy}-ethoxy}-acetic acid-2-Cl-Trt-Resin)-OtBu
Octadecanedioic acid mono thenyl ester
DIPC +HOBt
(Intermediate 2)
Trifluoroethanol/DCM (1:1)
IBCF+NMM+HOSU
TFA
(Intermediate 3)

**Figure 1B**

2-Cl-Trt-Resin + 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid

↓ DIPEA

2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

{2-(2-(2-amino-ethoxy)-ethoxy}-acetic acid-2-Cl-Trt-Resin

↓ 4-Nitropheylchloroformate + DIPEA

↓ 4-Fmoc-amino butylamine hydrochloride salt

↓ DIPC +HOBt

2-[2-[2-(4-Fmoc-aminobutylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

↓ Fmoc-Glu-OtBu +DIPC+HOBt

2-[2-[2-[4-[[(4S)-4-Fmoc-amino-5-tert-butoxy-5-oxo-pentanoyl]amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

↓ Octadecanedioic acid mono *tert*-butyl ester +DIPC+HOBt

2-[2-[2-[4-[[(4*S*)-5-*tert*-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

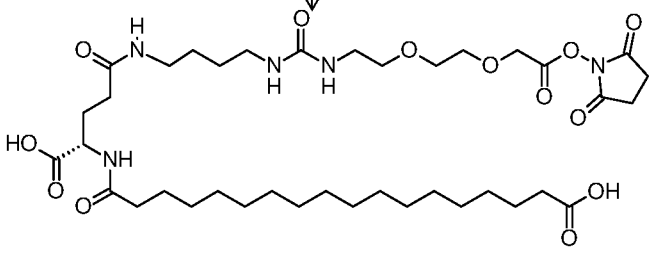
↓ Trifluoroethanol:DCM (1:1)

2-[2-[2-[4-[[(4*S*)-5-tert-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]butylcarbamoylamino]ethoxy]ethoxy]acetic acid

↓ DCC+HOSu

*tert*-butyl 18-[[(1*S*)-1-tert-butoxycarbonyl-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]butylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoate

↓ TFA



**Moiety C-OSu**

**Figure 2**

2-Cl-Trt-Resin + 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid

↓ DIPEA

2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-(2-aminoethoxy)ethoxy]acetic acid -2-Cl-Trt-Resin

↓ Fmoc-Aib-OH
DIPC +HOBt

2-[2-[2-[(2-Fmoc-amino-2-methyl-propanoyl)amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine
Fmoc-Glu-OtBu +DIPC+HOBt

2-[2-[2-[[2-[[(4*S*)-4-Fmoc-amino-5-*tert*-butoxy-5-oxo-pentanoyl]amino]-2-methyl-propanoyl] amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF
Octadecanedioic acid mono *tert* butyl ester +DIPC+HOBt

2-[2-[2-[[2-[[(4*S*)-5-*tert*-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl] amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

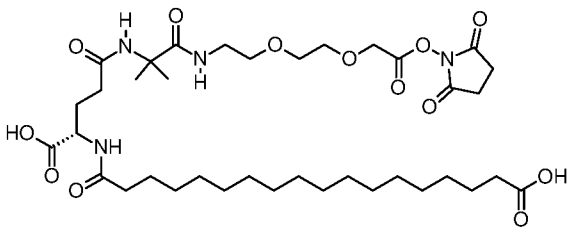
↓ Trifluoroethanol:DCM (1:1)

2-[2-[2-[[2-[[(4*S*)-5-tert-butoxy-4-[(18-*tert*-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl] amino]-2-methyl-propanoyl]amino]ethoxy]ethoxy]acetic acid

↓ DCC+HOSu

tert-butyl 18-[[(1S)-1-tert-butoxycarbonyl-4-[[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylamino]-1,1-dimethyl-2-oxo-ethyl]amino]-4-oxo-butyl]amino]-18-oxo-octadecanoate

↓ TFA



**Moiety D-OSu**

**Figure 3**

2-Cl-Trt-Resin + 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid

↓ DIPEA

2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid -2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-(2-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 4-Nitropheylchloroformate + DIPEA
1,3-Diaminopropane +HOBt
Fmoc-Glu-OtBu +DIPC+HOBt

2-[2-[2-[3-[[(4S)-4-Fmoc-amino-5-tert-butoxy-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF
Octadecanedioic acid mono *tert* butyl ester +DIPC+HOBt

2-[2-[2-[3-[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

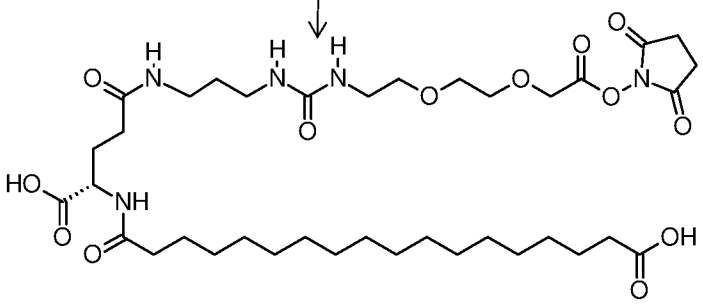
↓ Trifluoroethanol:DCM (1:1)

2-[2-[2-[3-[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]propylcarbamoylamino]ethoxy]ethoxy]acetic acid

↓ DCC+HOSu

tert-butyl 18-[[(1S)-1-tert-butoxycarbonyl-4-[3-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]propylamino]-4-oxo-butyl]amino]-18-oxo-octadecanoate

↓ TFA



**Moiety E-OSu**

**Figure 4**

2-Cl-Trt-Resin + 2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid

↓ DIPEA

2-[2-(2-Fmoc-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF

2-[2-(2-aminoethoxy)ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 4-Nitropheylchloroformate + DIPEA
↓ 4-Amino-Boc-piperidine
↓ DIPEA +HOBt

(2-[2-[2-(4-Boc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid -2-Cl-Trt-Resin

↓ TFA

2-[2-[2-(4-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid

↓ Fmoc-OSu, TEA

2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid + 2-Cl-Trt-Resin

↓ DIPEA

2-[2-[2-(4-Fmoc-piperidylcarbamoylamino)ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF
↓ Fmoc-Glu-OtBu +DIPC+HOBt

2-[2-[2-[[1-[(4S)-4-amino-5-tert-butoxy-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

↓ 50% Piperidine in DMF
↓ Octadecanedioic acid mono *tert* butyl ester +DIPC+HOBt

2-[2-[2-[[1-[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid-2-Cl-Trt-Resin

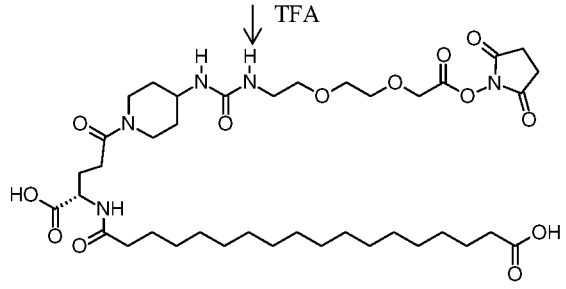
↓ Trifluoroethanol:DCM (1:1)

2-[2-[2-[[1-[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]-4-piperidyl]carbamoylamino]ethoxy]ethoxy]acetic acid

↓ DCC+HOSu

tert-butyl 18-[[(1S)-1-tert-butoxycarbonyl-4-[4-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylcarbamoylamino]-1-piperidyl]-4-oxo-butyl]amino]-18-oxo-octadecanoate

↓ TFA



**Moiety F-OSu**

**Figure 5**

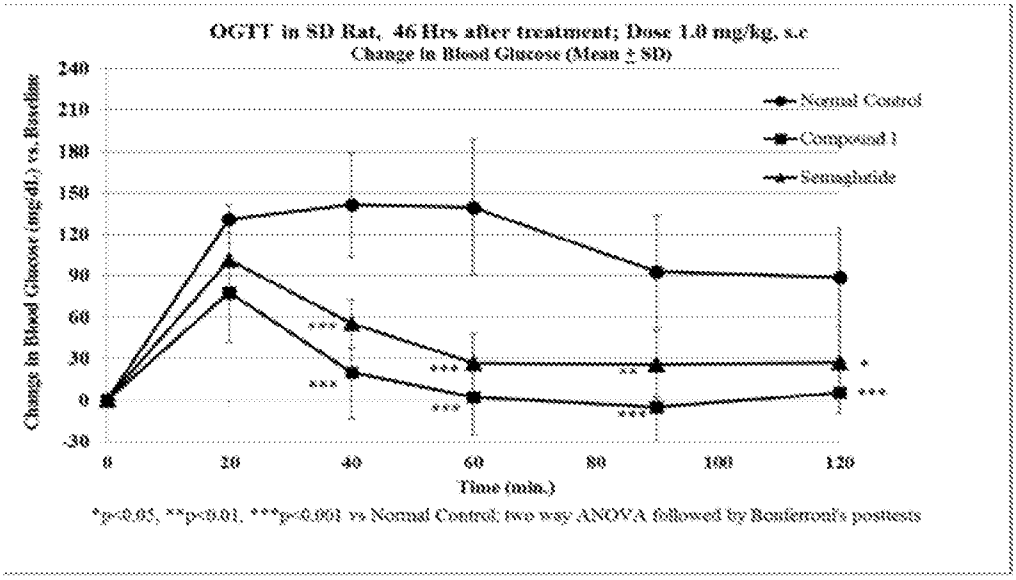



Figure 6 (A)

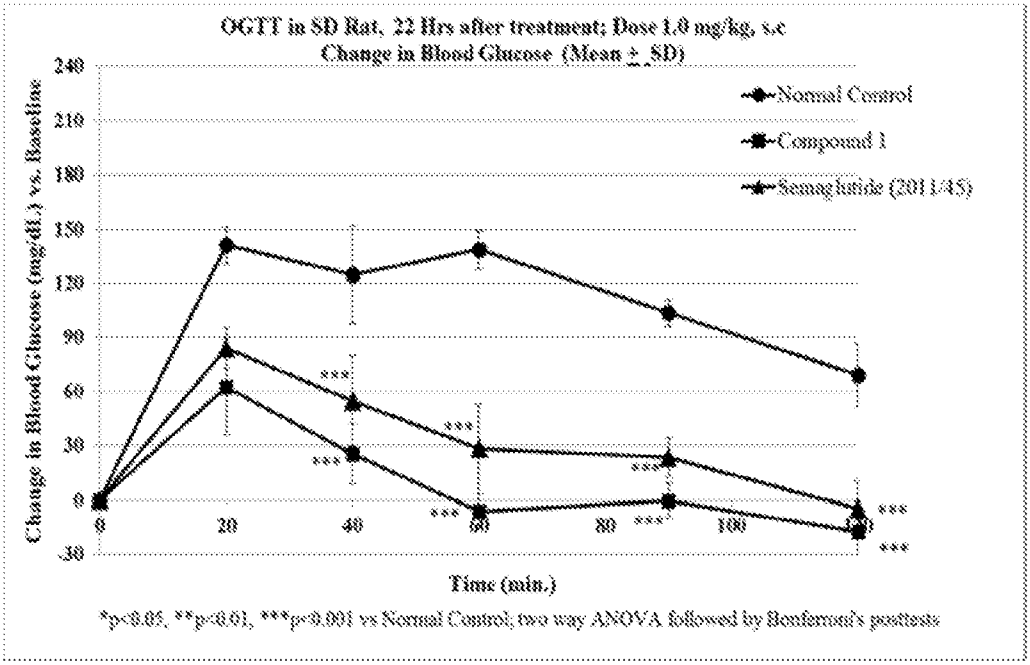



Figure 6 (B)

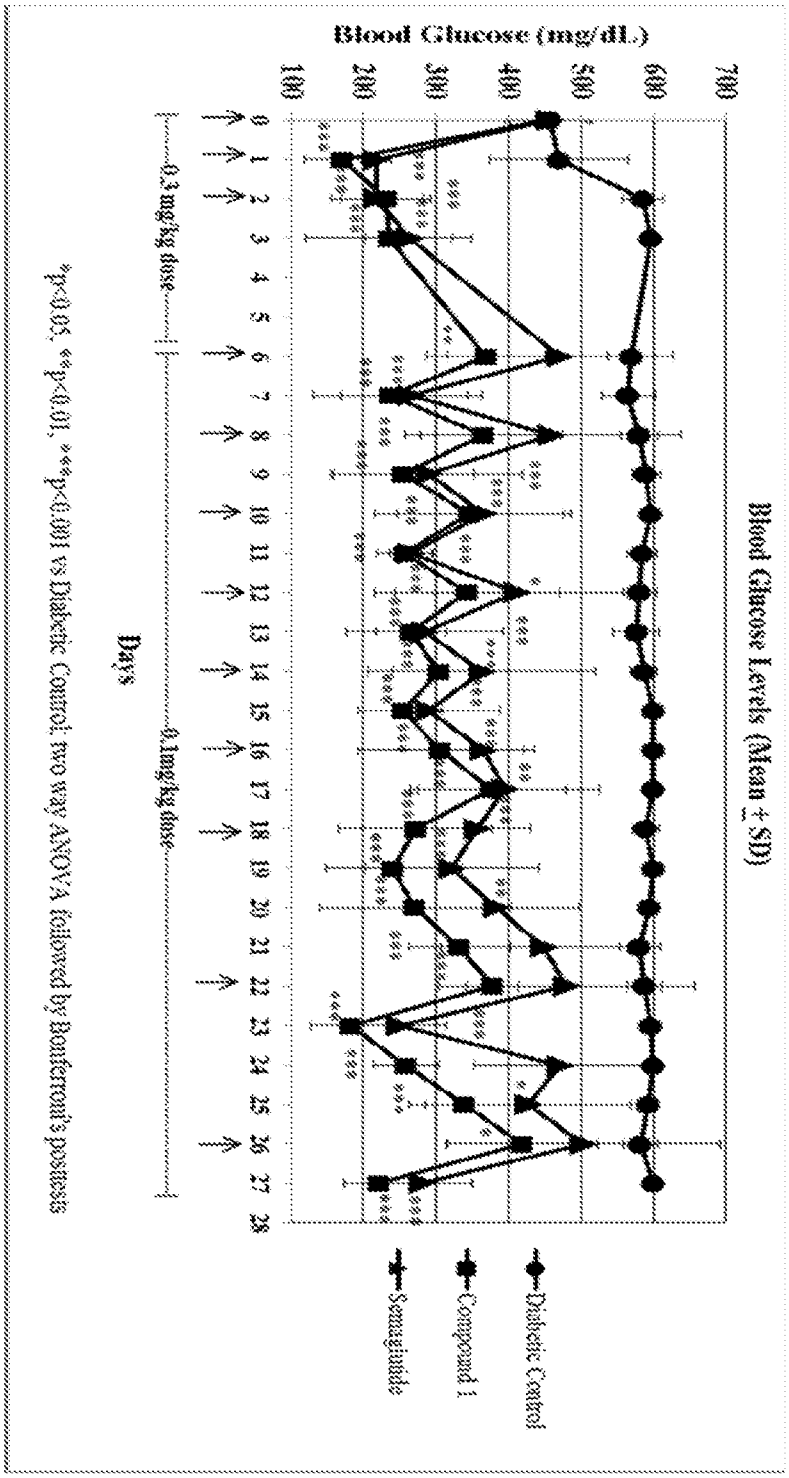
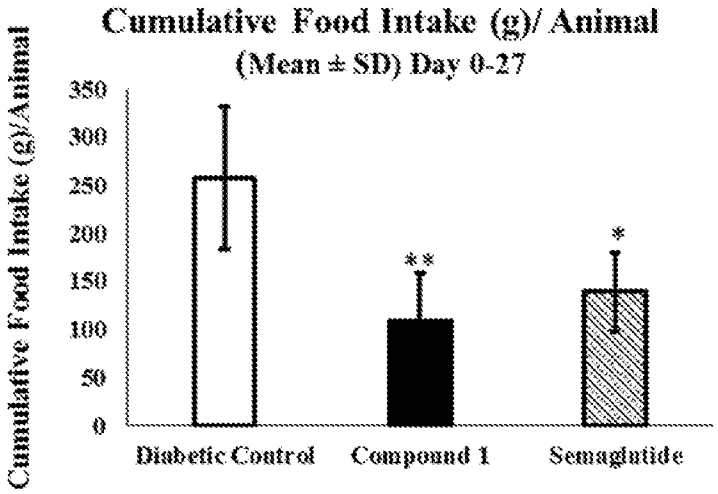

Blood Glucose (mg/dL)
100
200
300
400
500
600
700
Blood Glucose Levels (Mean ± SD)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
0.3 mg/kg dose
0.1mg/kg dose
Days
Semaglutide
Compound 1
Diabetic Control
*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control; two way ANOVA followed by Bonferroni's posttest

Figure 7




*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control; one way ANOVA followed by Dunnett's posttests

Figure 8

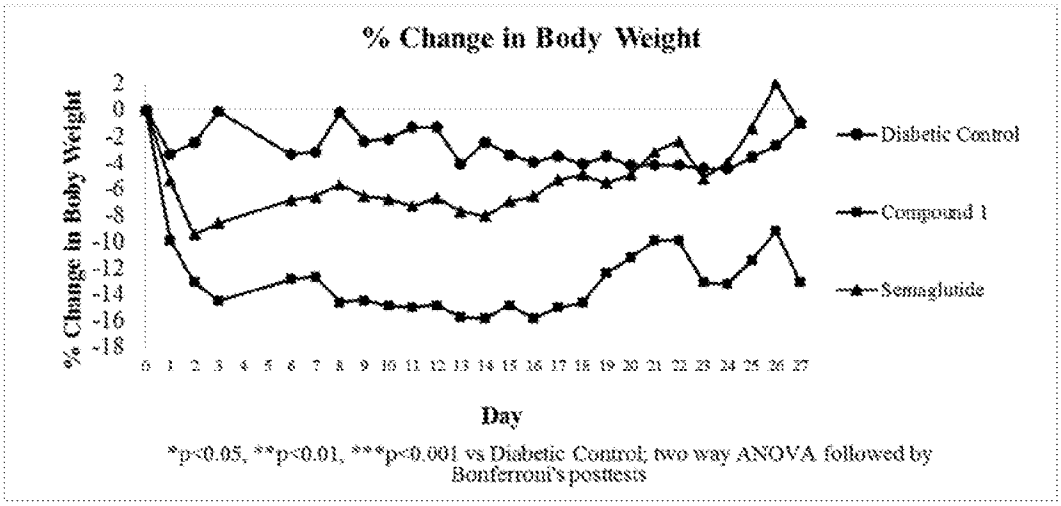
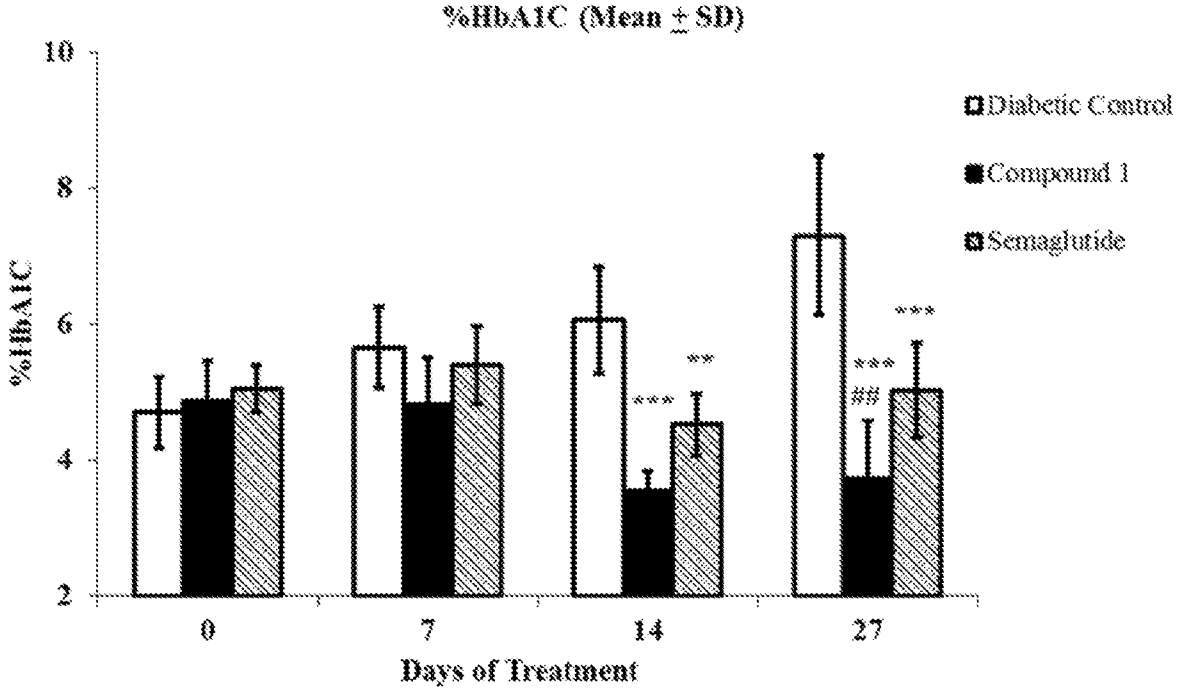

% Change in Body Weight
% Change in Body Weight
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Day
Diabetic Control
Compound 1
Semaglutide
*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control; two way ANOVA followed by Bonferroni's posttests

Figure 9




*p<0.05, **p<0.01, ***p<0.001 vs Diabetic Control and #p<0.05, ##p<0.01, ###p<0.001 vs Semaglutide; two way ANOVA followed by Bonferroni's posttests

Figure 10