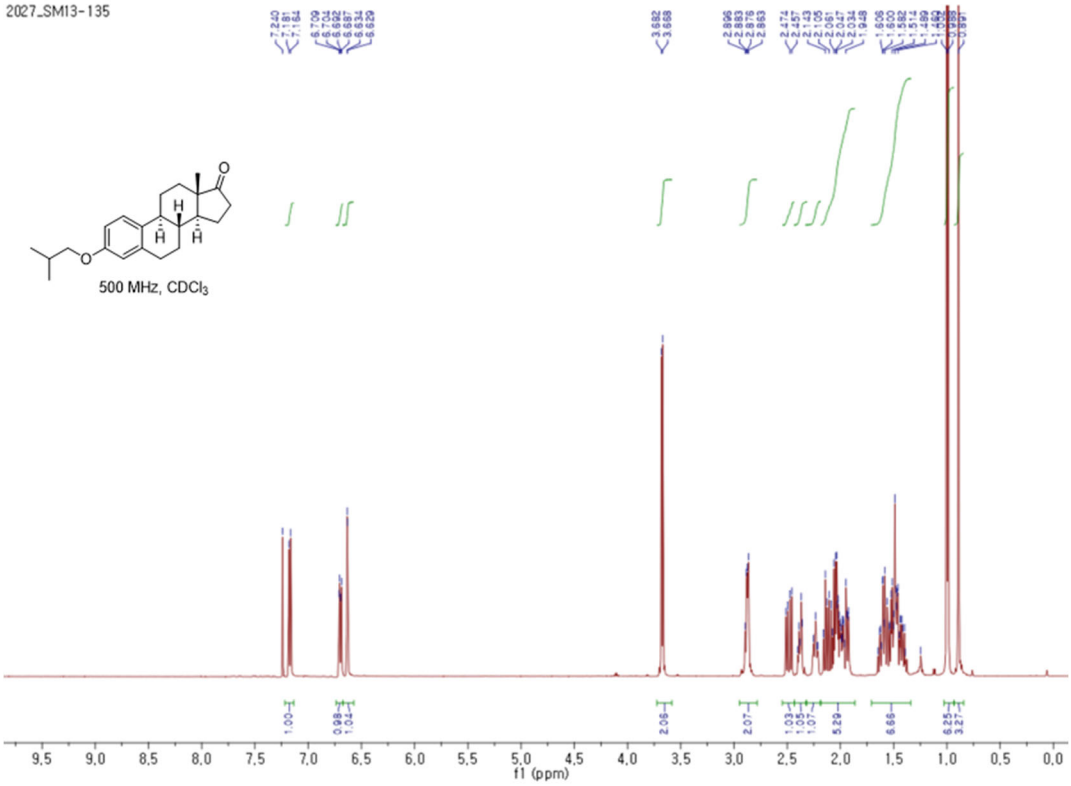
iScience, Volume *27*

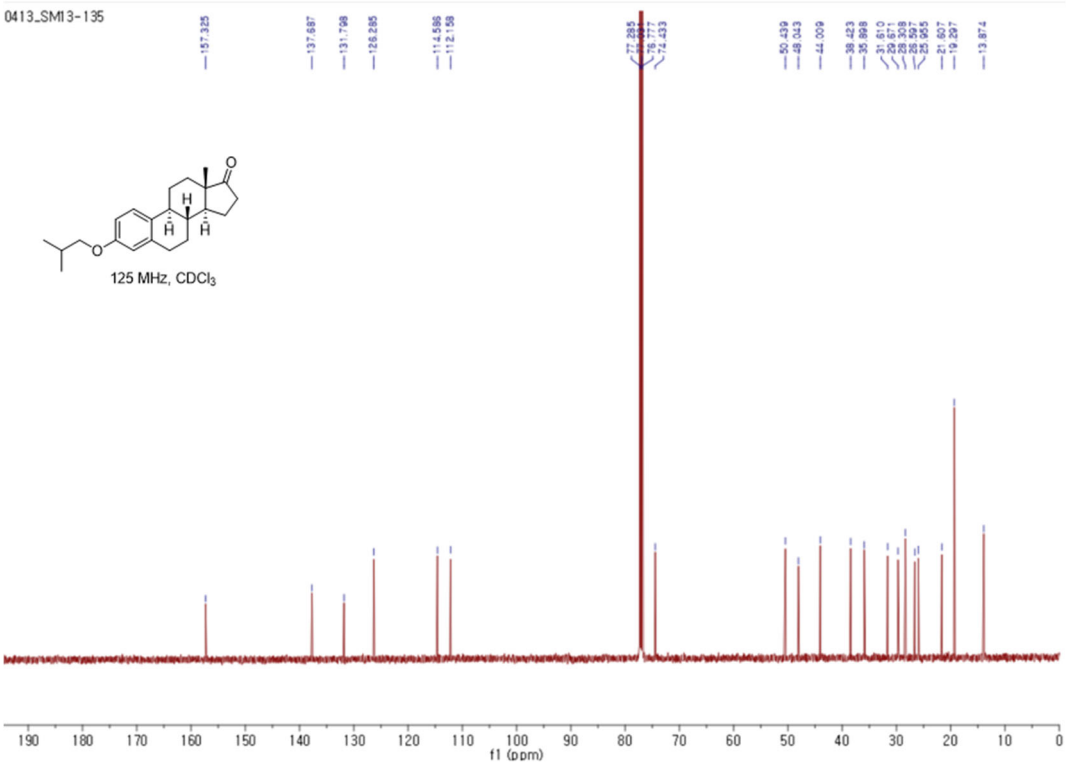
Supplemental information

Dual inhibition of aminoacyl-tRNA synthetase interacting multifunctional protein-2 and α-synuclein by steroid derivative is neuroprotective in Parkinson's model

Jeong-Yong Shin, Min Woo Ha, Ji Hun Kim, Jiwon Cheon, Gum Hwa Lee, Seung-Mann Paek, and Yunjong Lee

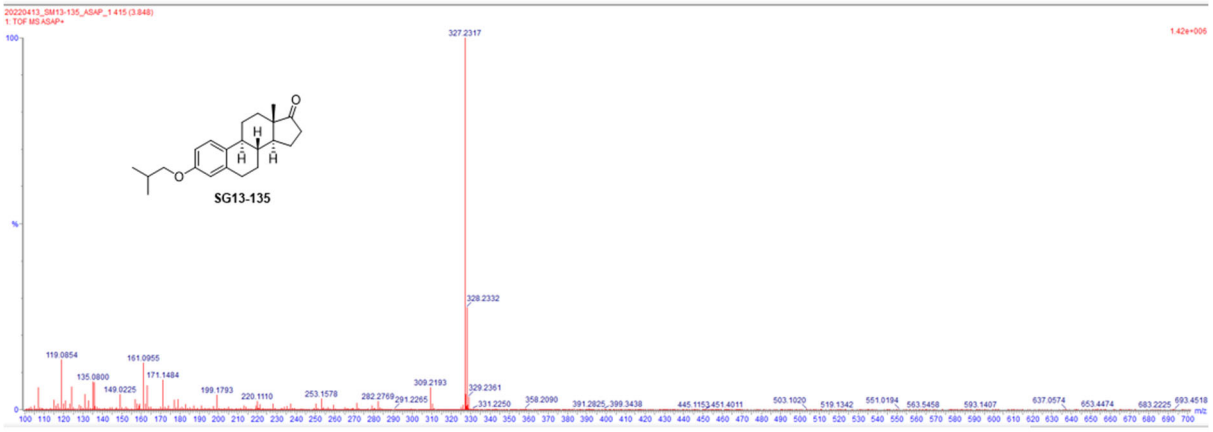
<Spectral data of SG13-135>

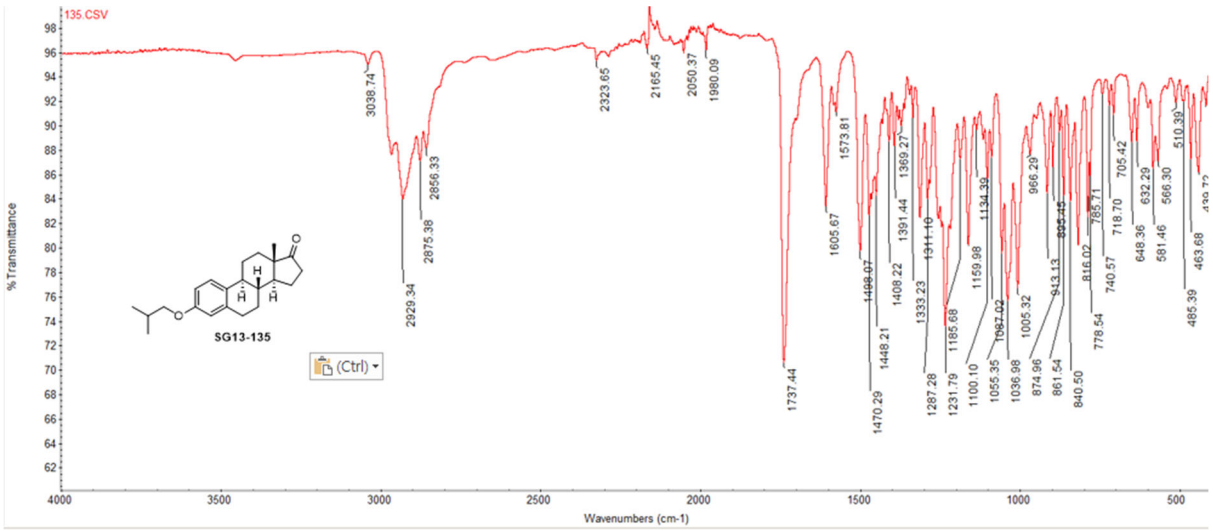




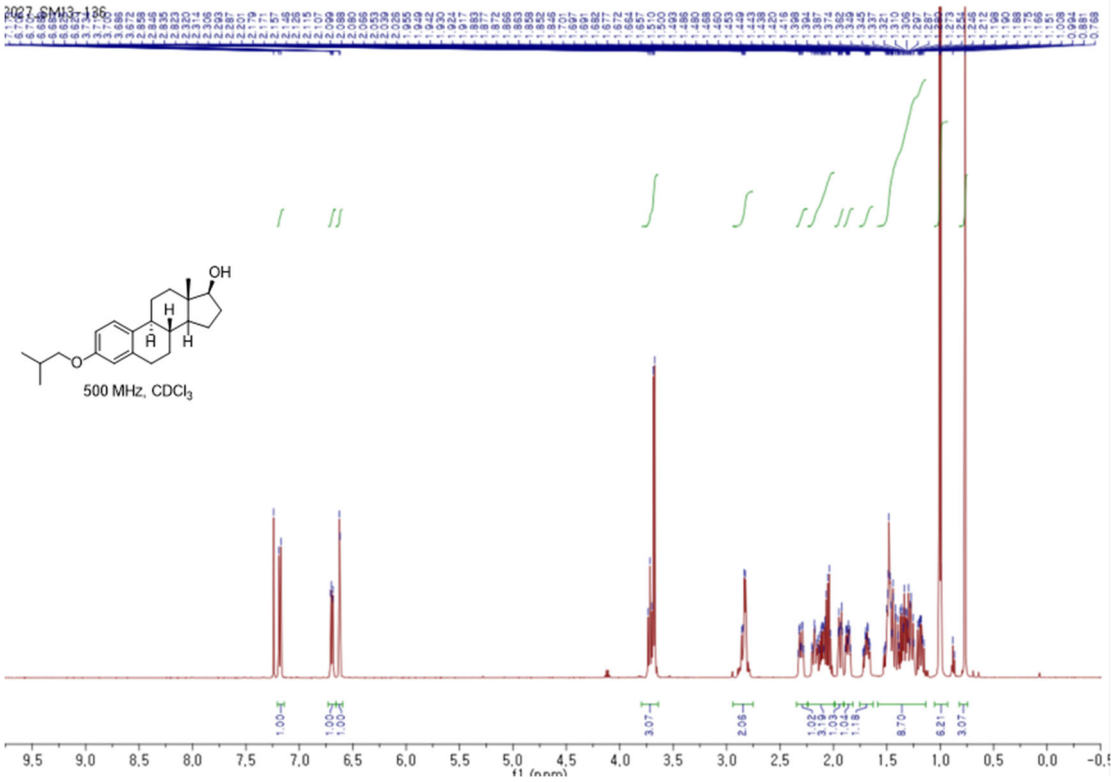
Single Mass Analysis
Tolerance = 30.0 PPM / DBE: min = -3.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9
Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:

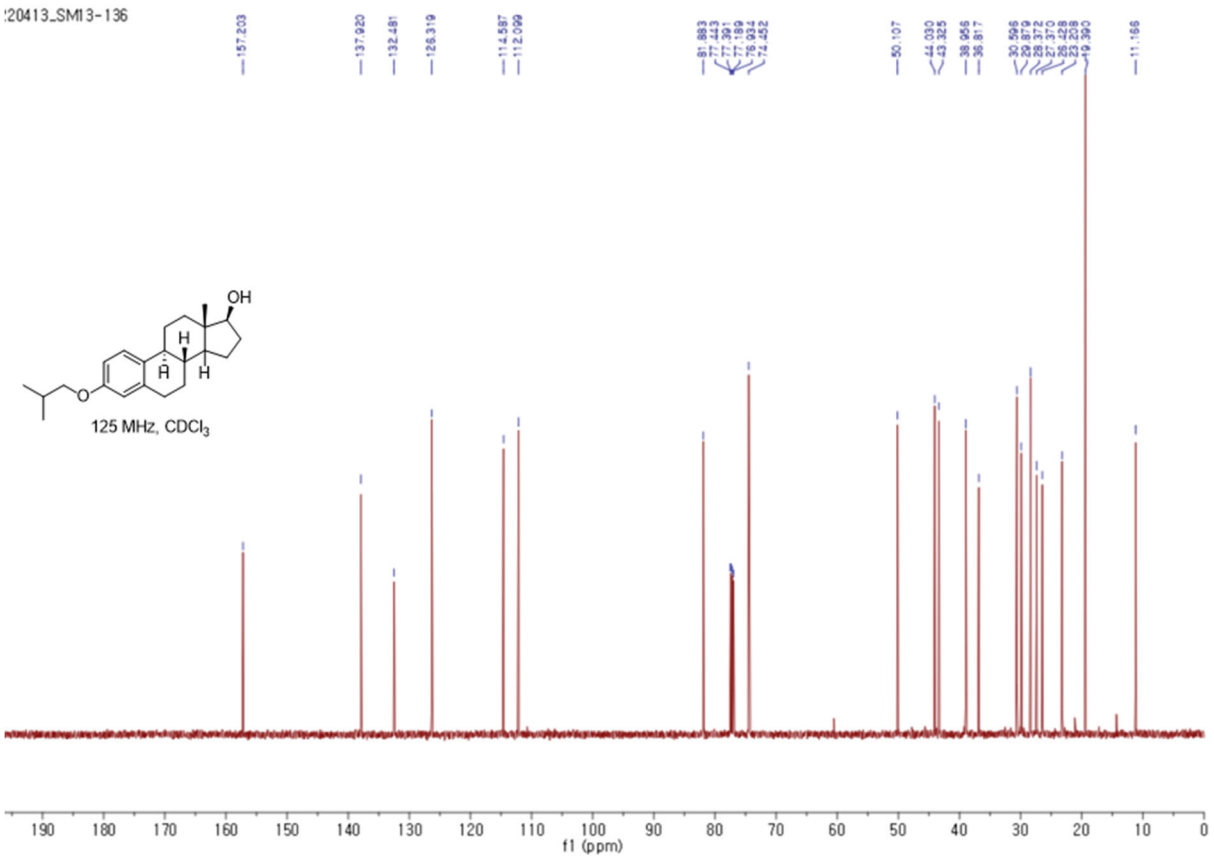
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
327.2317	327.2324	-0.7	-2.1	7.5	C22 H31 O2	1741.6	n/a	n/a	22	31	2





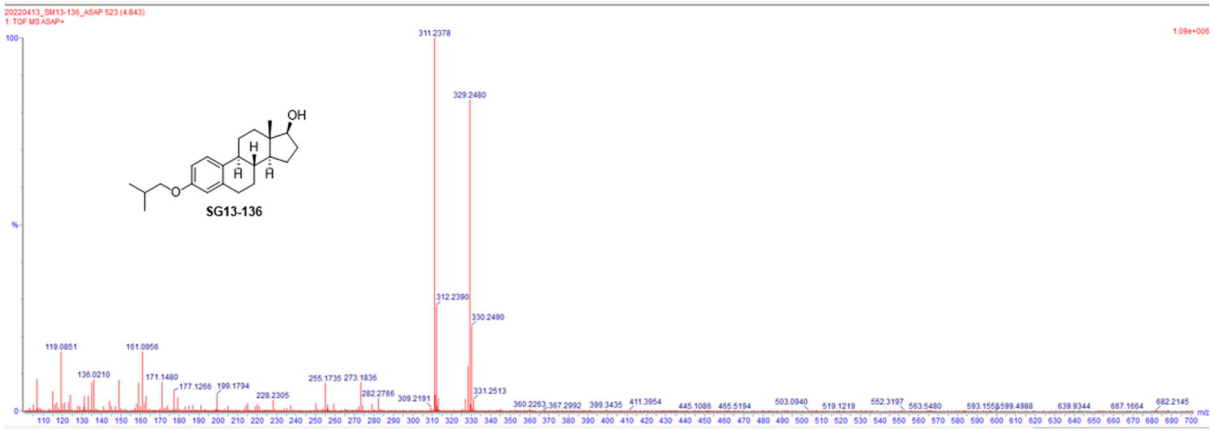
<Spectral data of SG13-136>

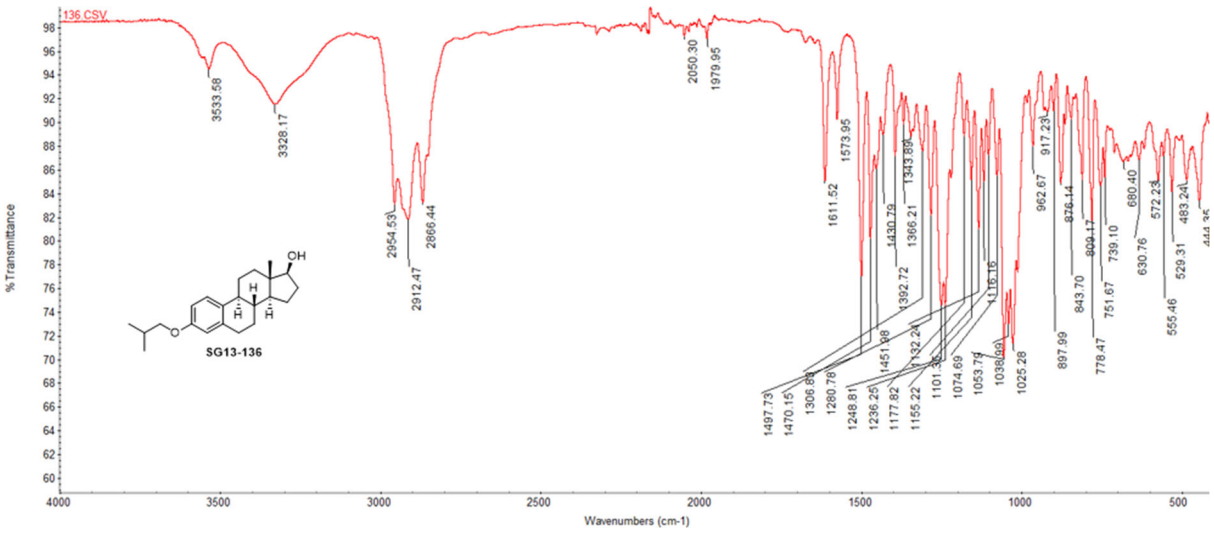




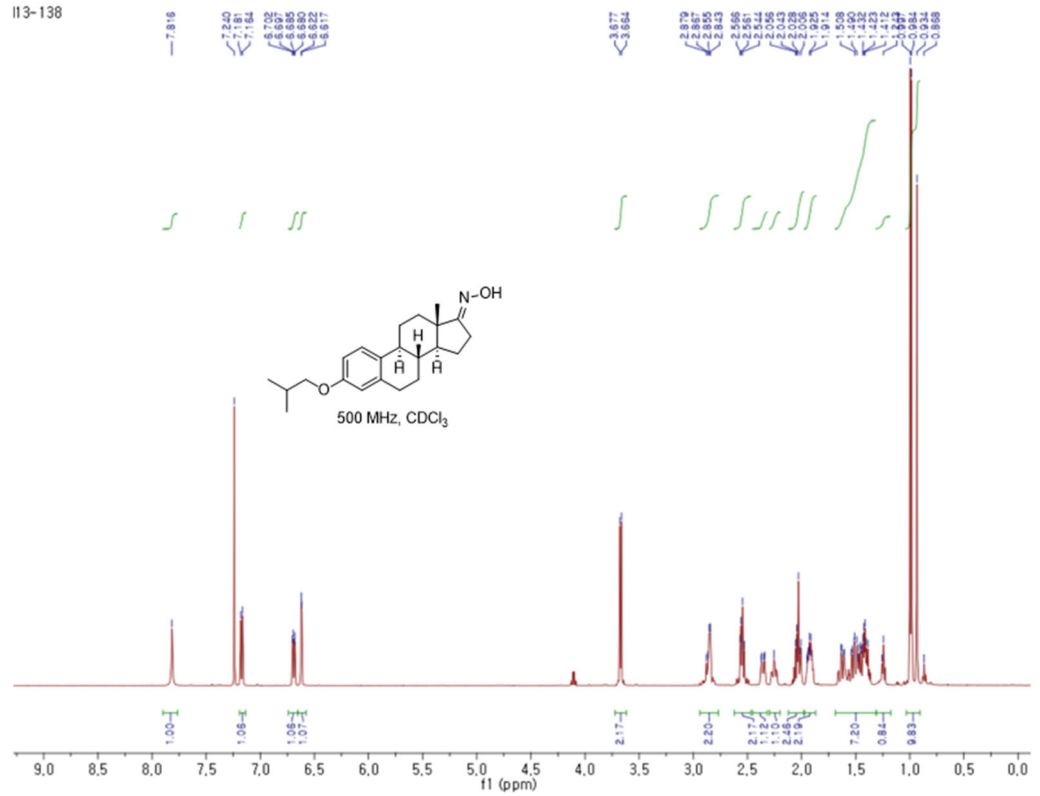
Single Mass Analysis
Tolerance = 30.0 PPM / DBE: min = -3.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9
Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:

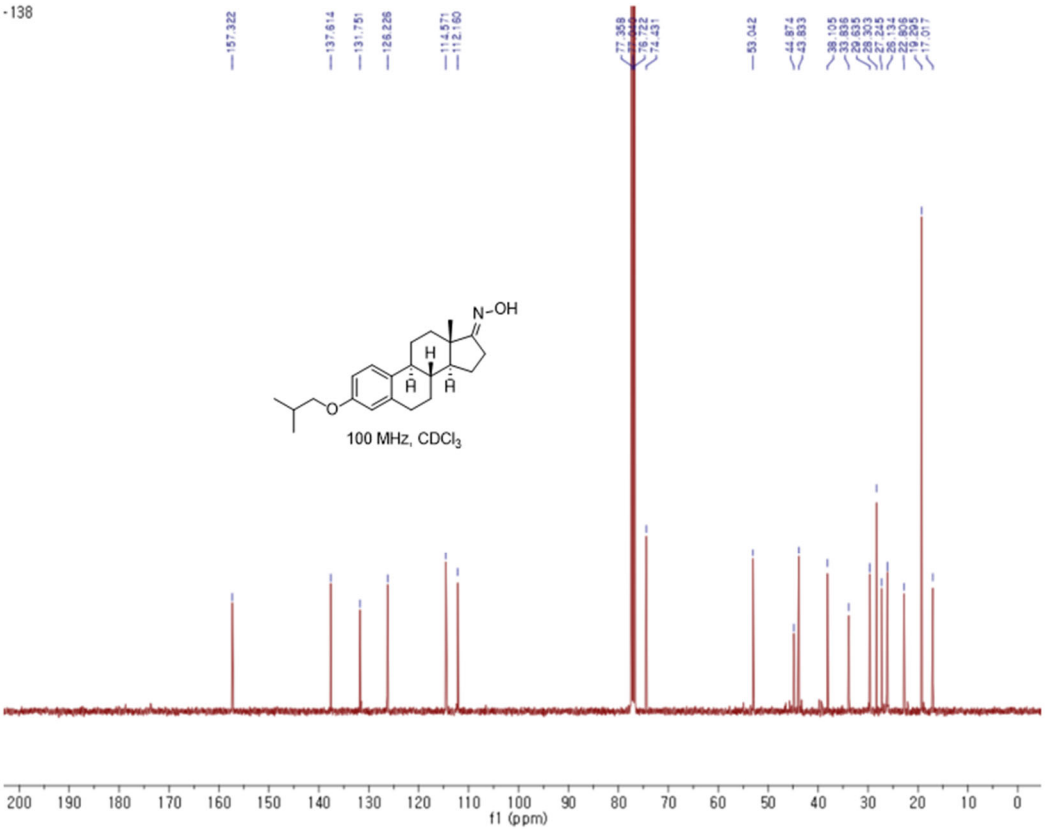
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
329.2480	329.2481	-0.1	-0.3	6.5	C22 H33 O2	1503.2	n/a	n/a	22	33	2





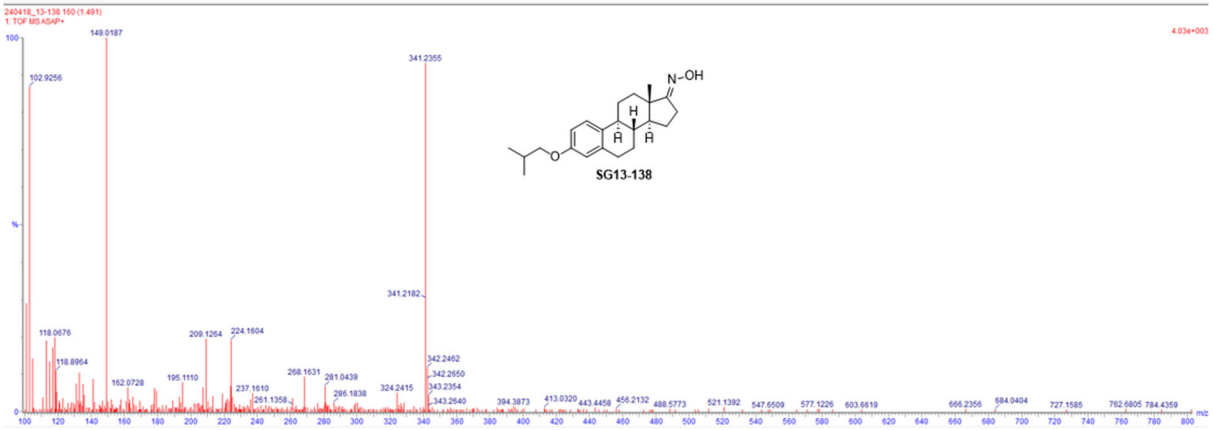
<Spectral data of SG13-138>

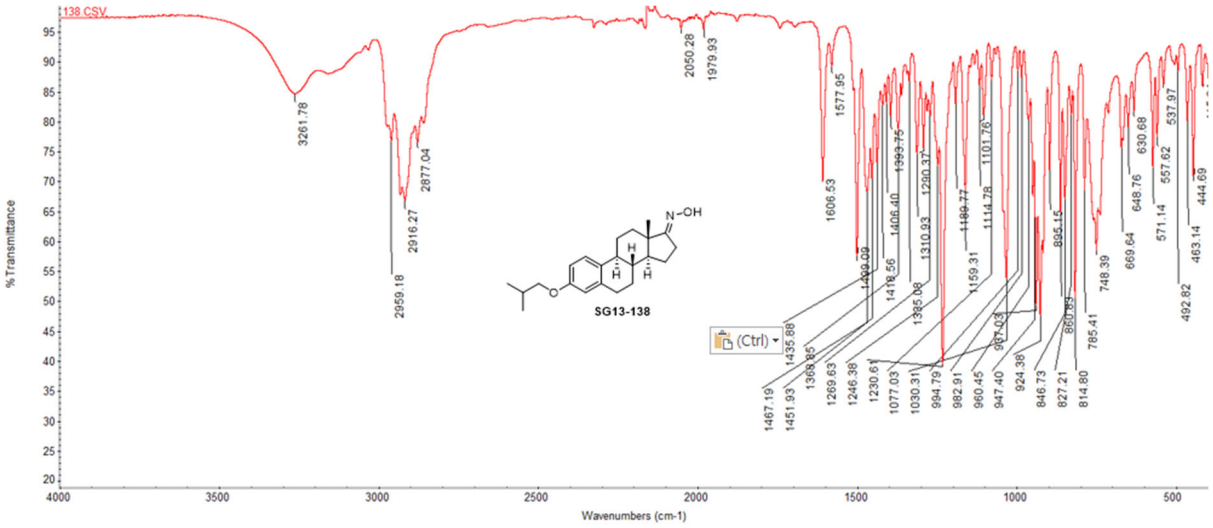




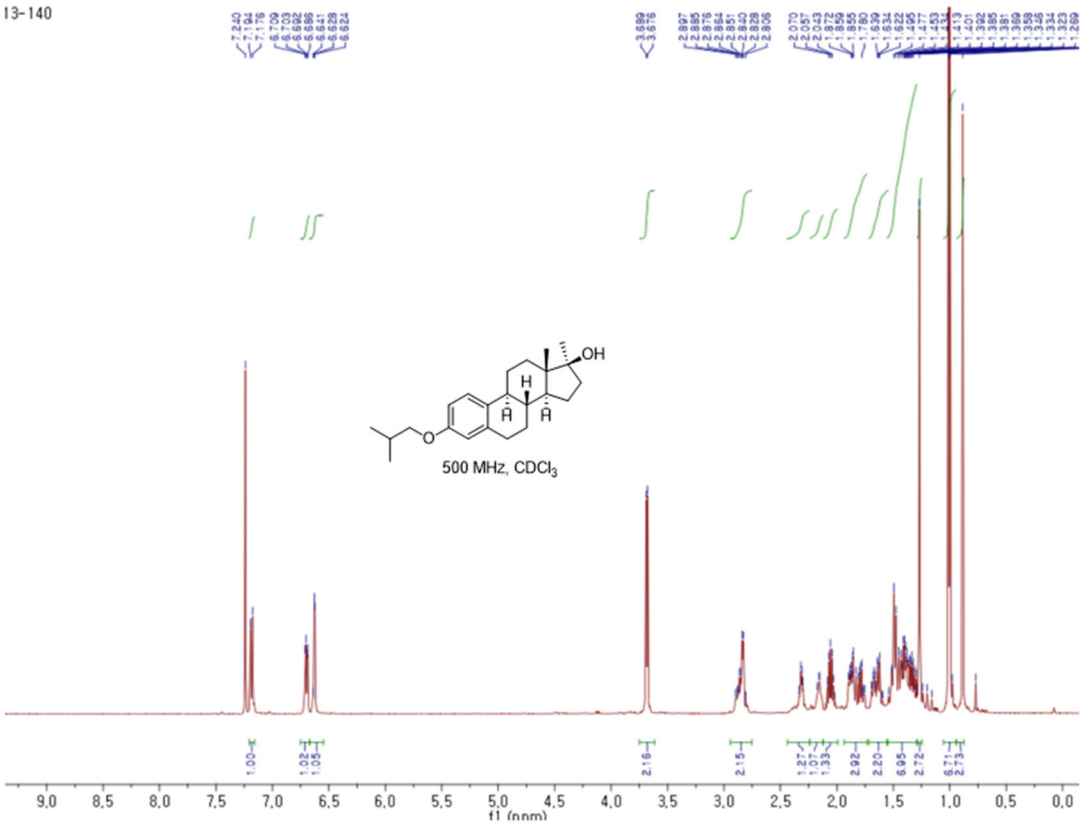
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -30.0, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Odd and Even Electron Ions
102 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:

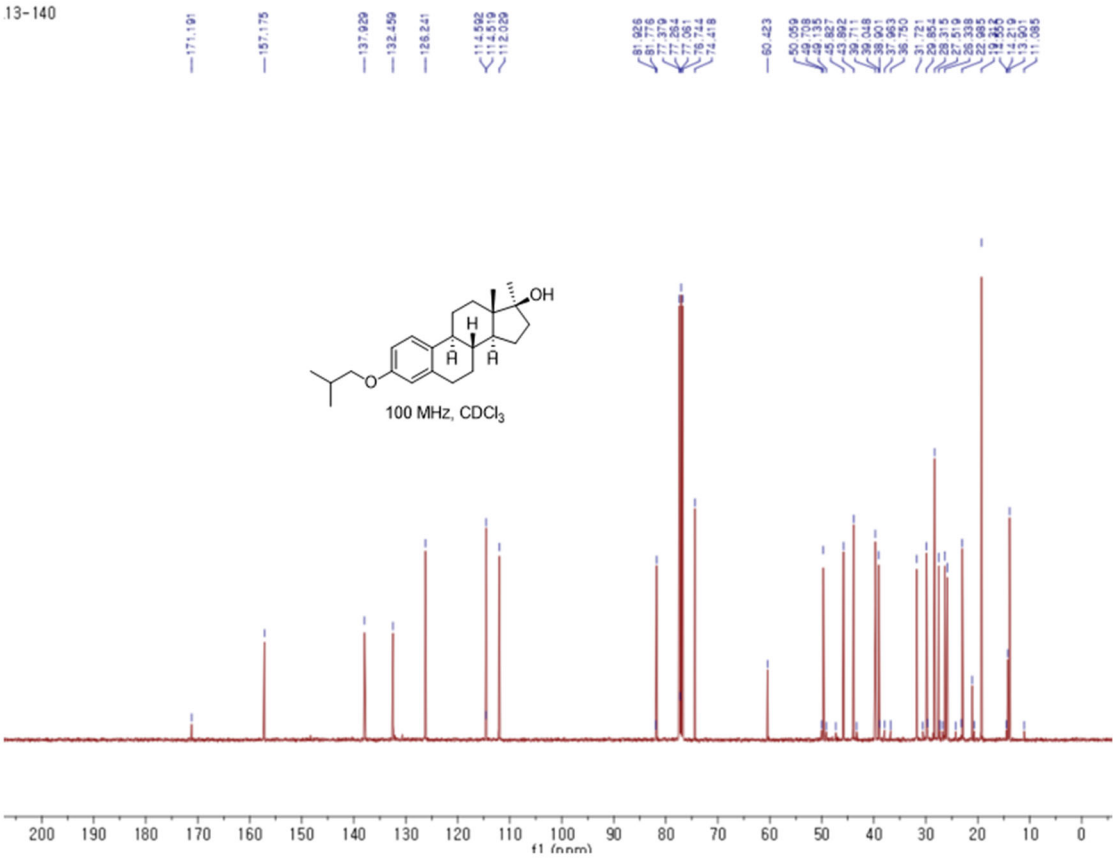
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
341.2355	341.2355	0.0	0.0	8.0	C22 H31 N O2	102.7	n/a	n/a	22	31	1	2





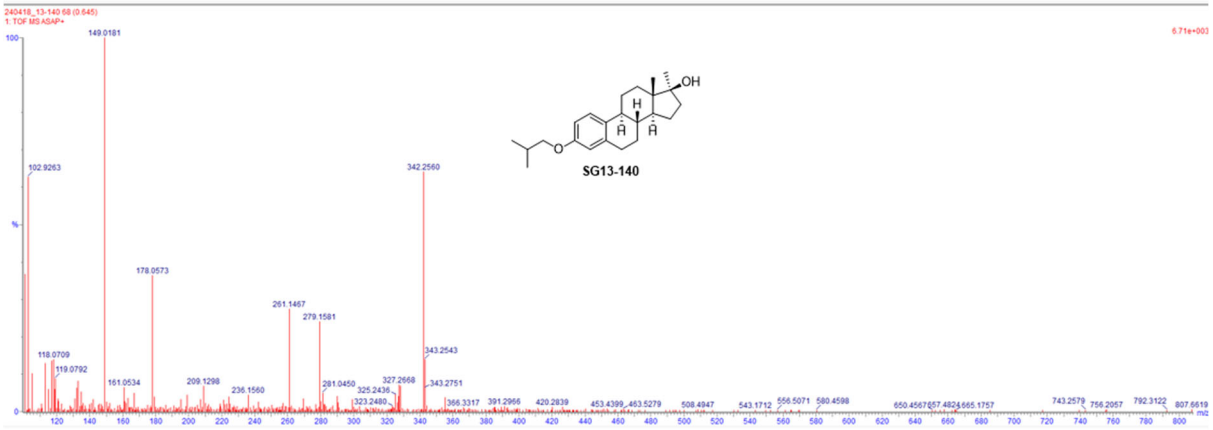
<Spectral data of SG13-140>

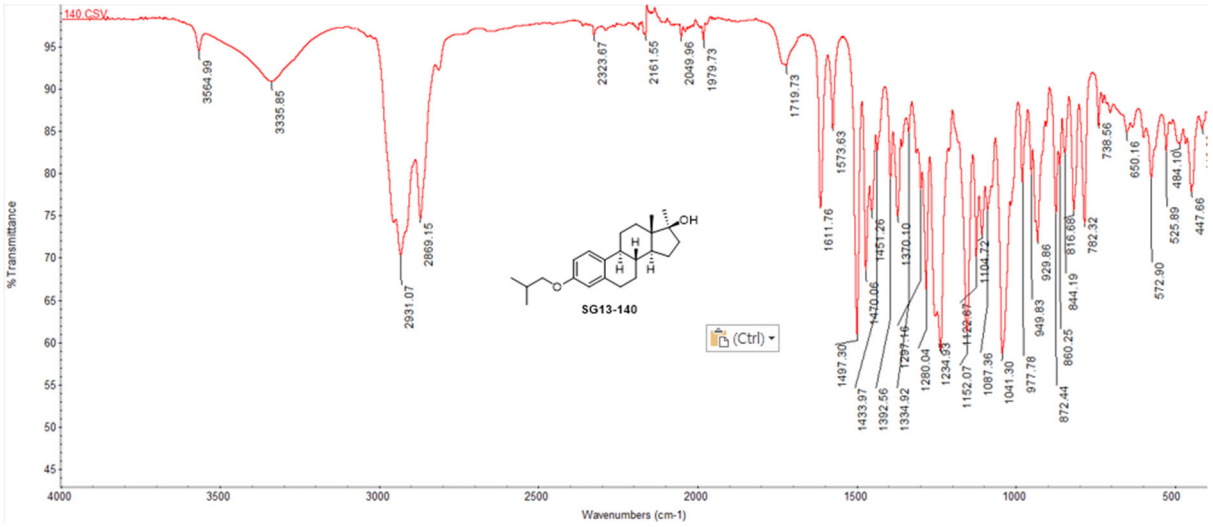




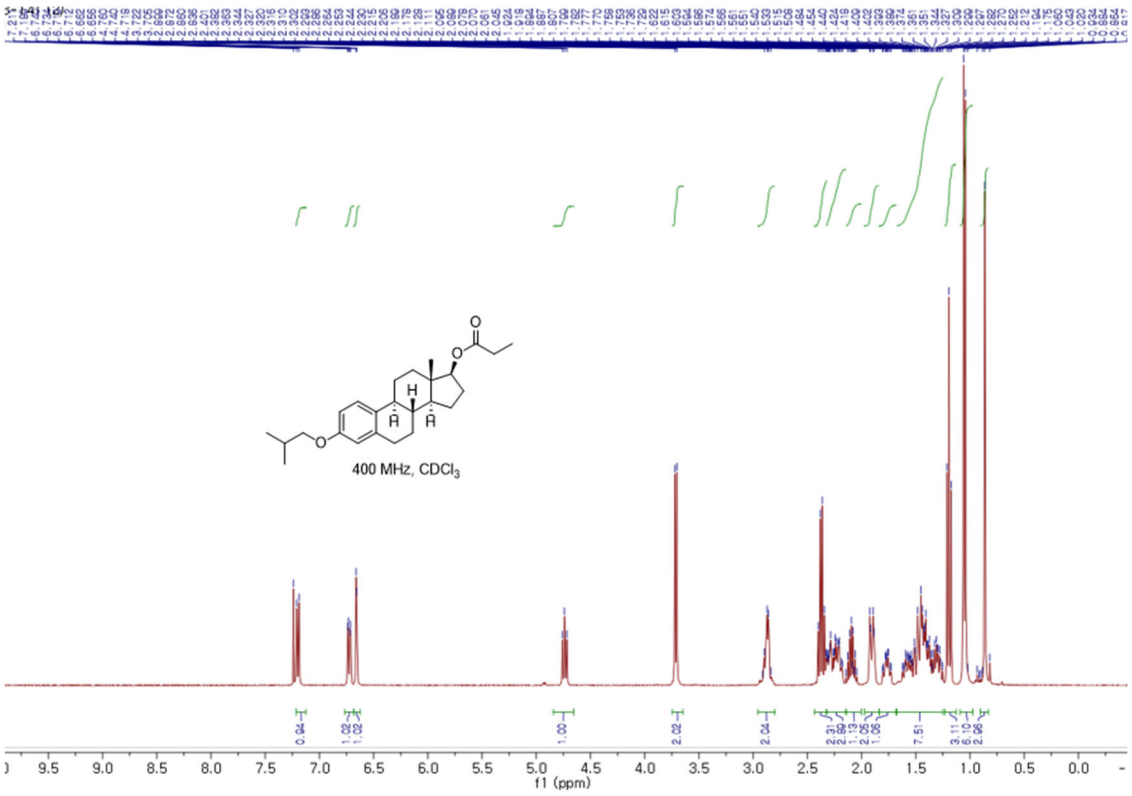
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -30.0, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Odd and Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:

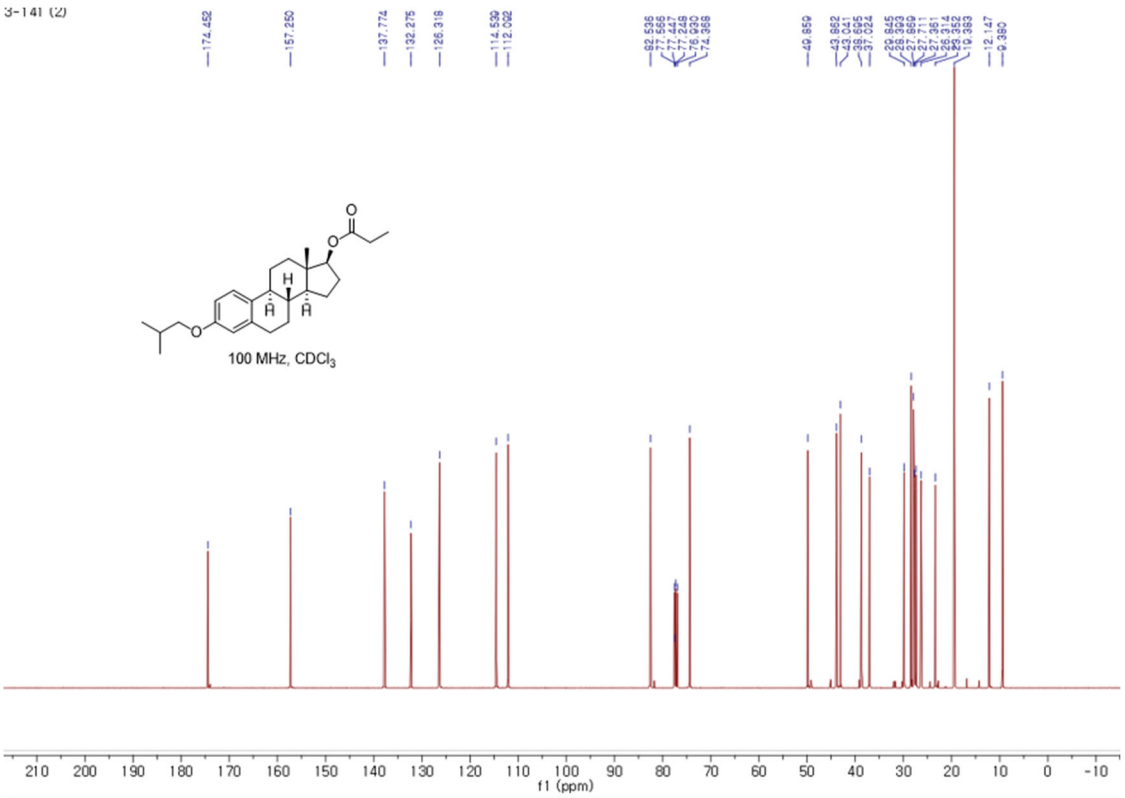
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
342.2560	342.2559	0.1	0.3	7.0	C23 H34 O2	93.8	n/a	n/a	23	34	2





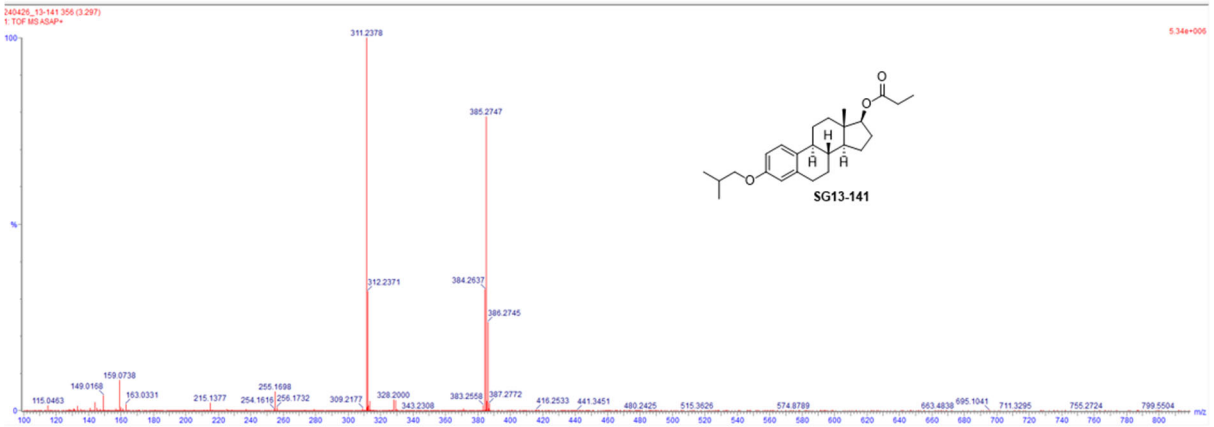
<Spectral data of SG13-141>

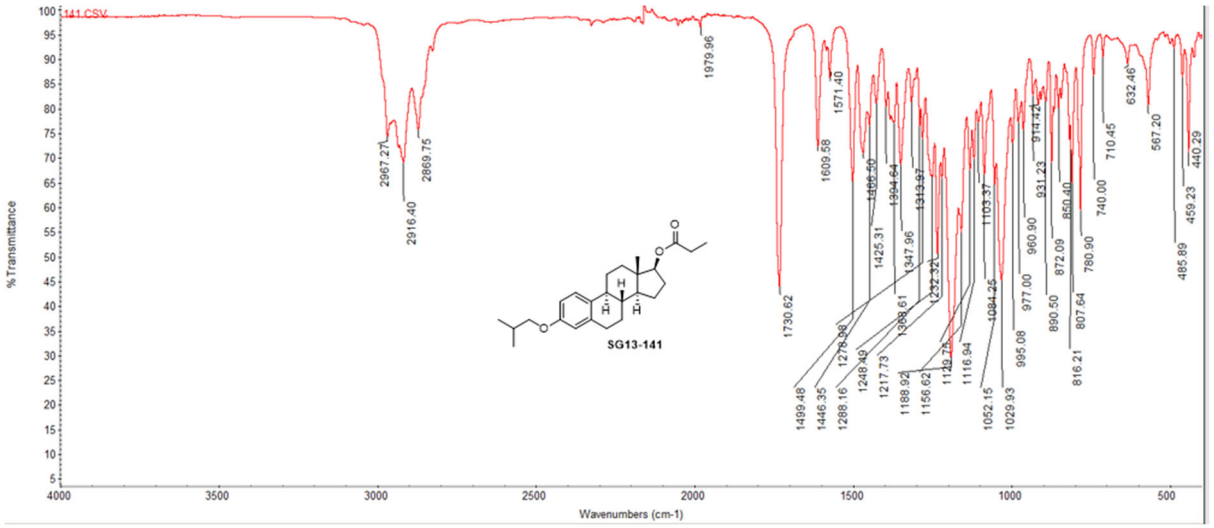




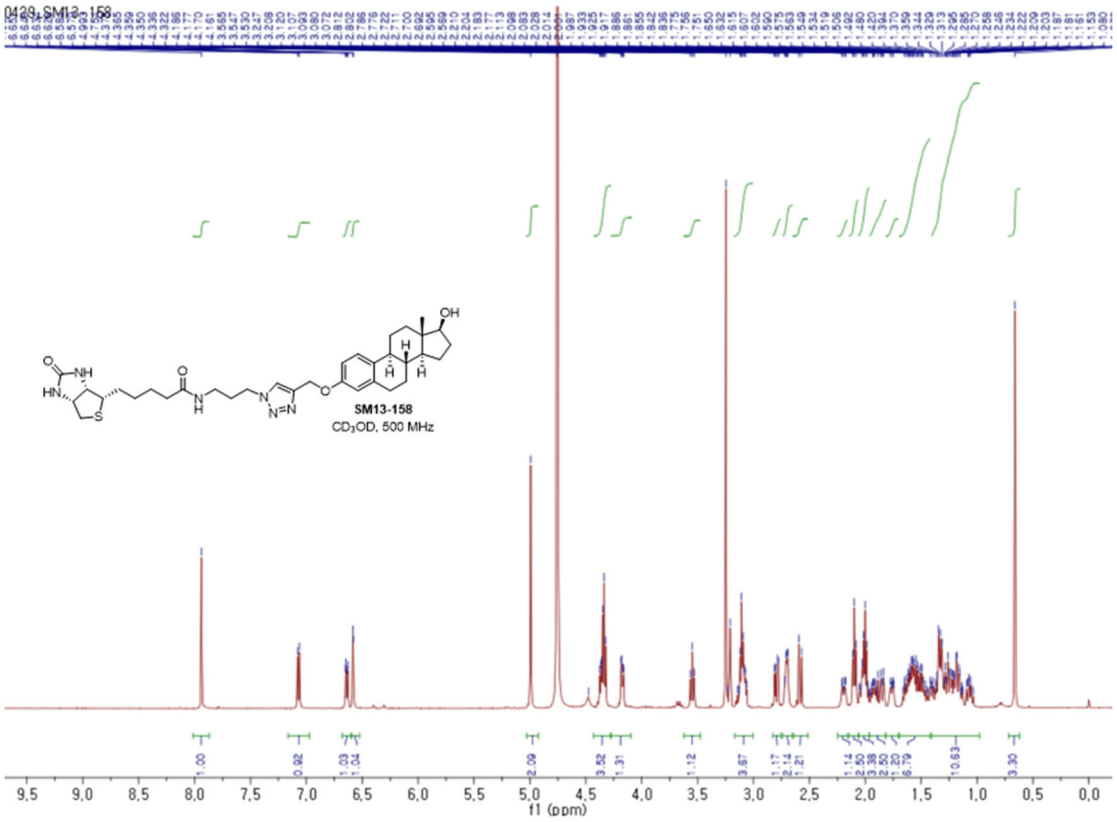
Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -30.0, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
42 formula(e) evaluated with 1 results within limits (up to 5 closest results for each mass)
Elements Used:

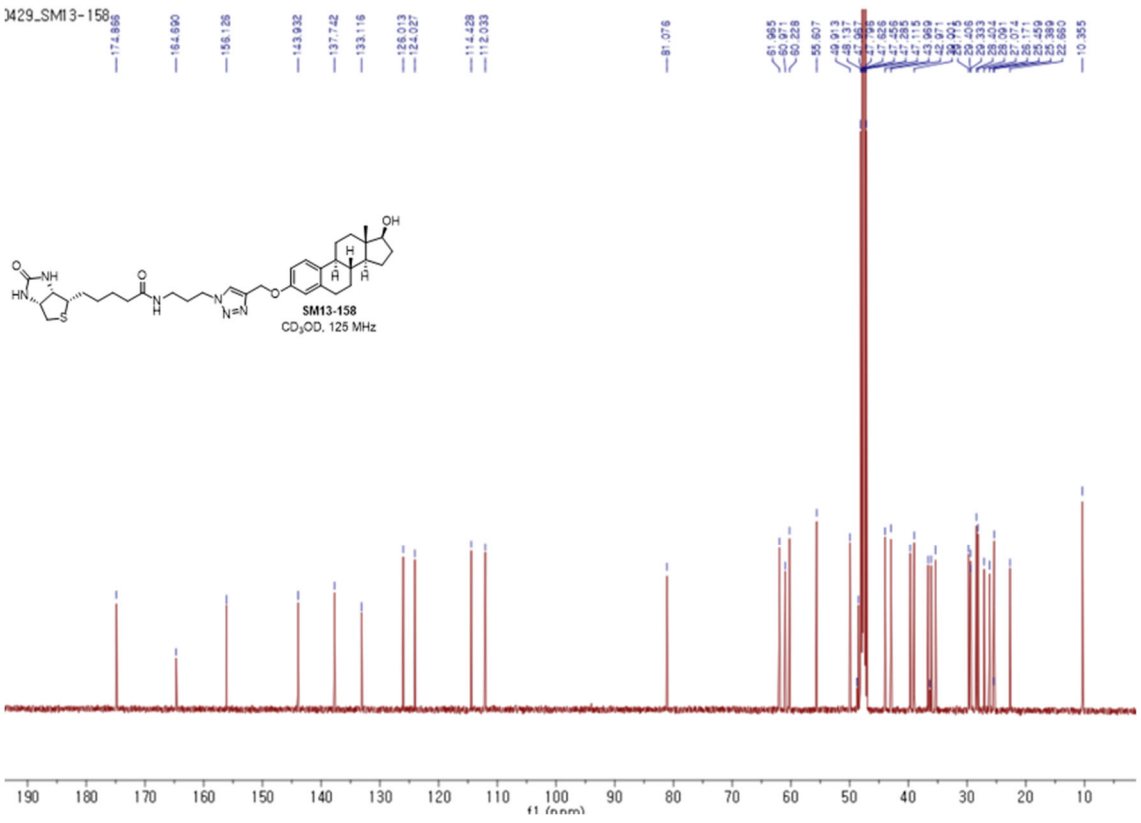
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
385.2747	385.2743	0.4	1.0	7.5	C25 H37 O3	538.5	n/a	n/a	25	37	3

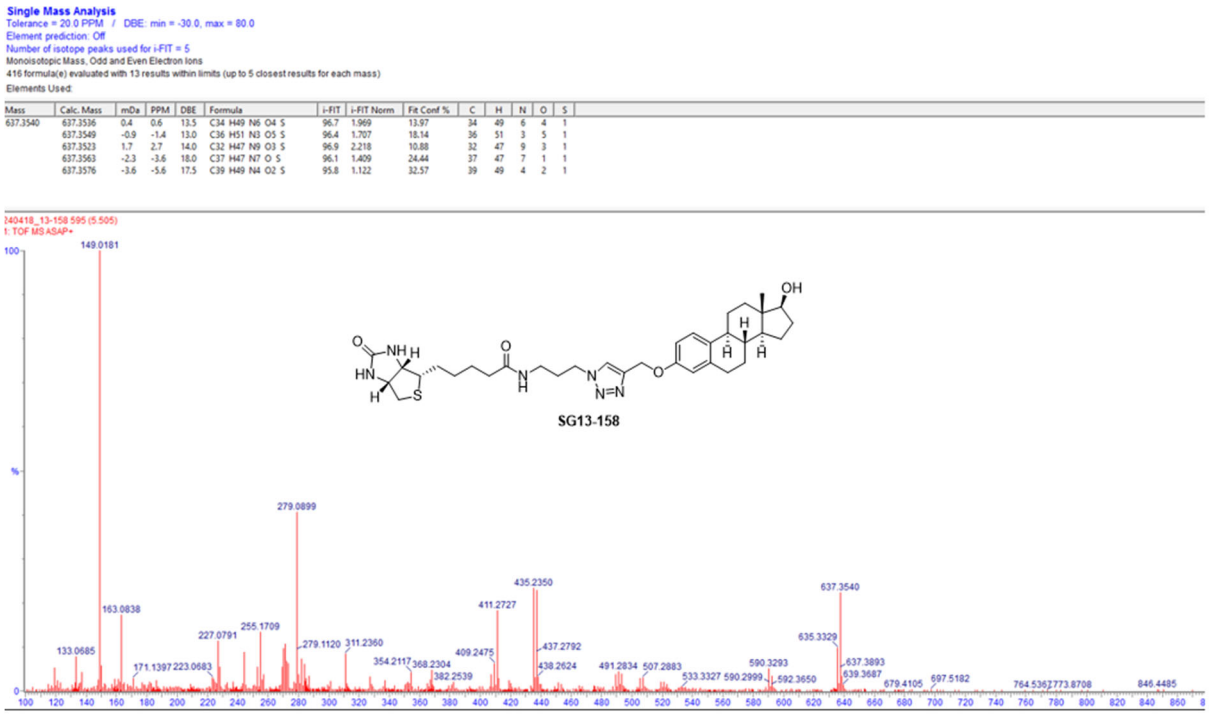




<Spectral data of SG13-158>

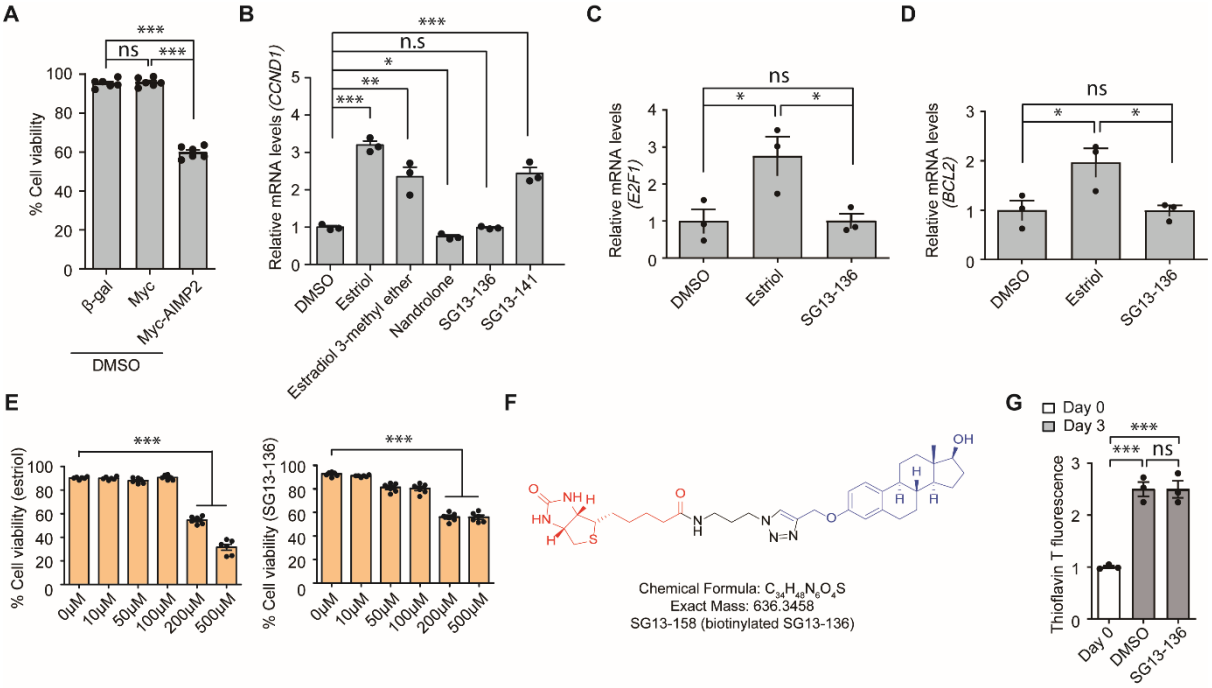






Data S1. Related to Figure 1. Chemical structures and ^{1}H-NMR spectra of newly synthesized steroid derivatives.

The ^{1}H-NMR spectra illustrate the chemical structures of designated compounds (SG13-135, SG-13-136, SG13-138, SG13-140, SG13-141, and SG13-158).



Supplementary Figure 1. Related to Figure 2 and 3. Reduction of cytotoxicity, absence of estrogenic activity, and no inhibition of Tau aggregation of SG13-136.

(A) Trypan blue exclusion cell viability assessment conducted in SH-SY5Y cells transfected with β-gal, Myc empty vector control, or Myc-AIMP2 (72 h) (n = 6 per group).

(B) Quantification of the estrogen responsive gene, *CCND1* messenger RNA levels in a human breast tumor cell line, MCF7, treated with the specified compounds (10 μM, 48 h), monitored by real-time quantitative PCR (n = 3 separate experiments per group). *GAPDH* served as an internal loading control for normalization.

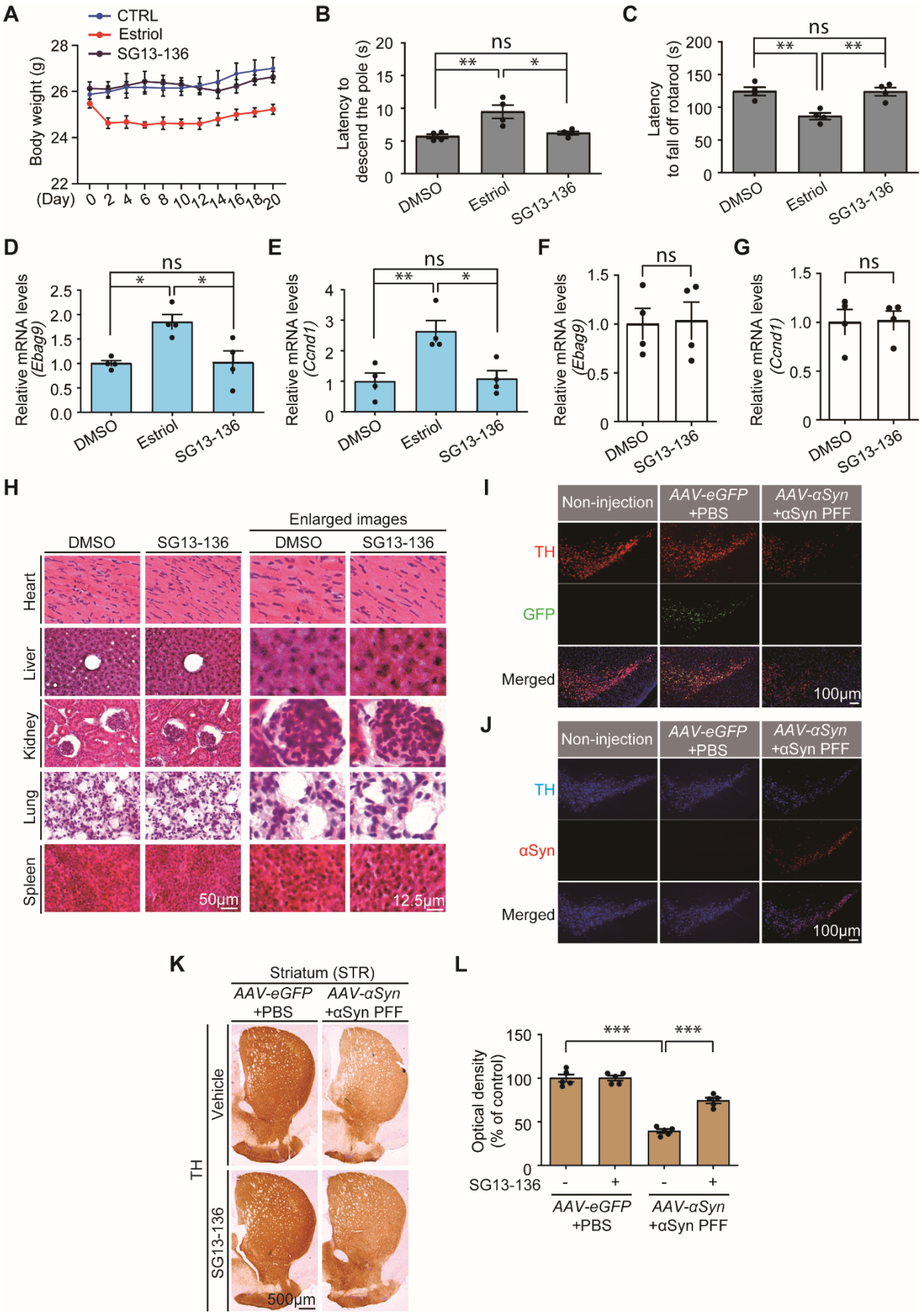
(C, D) Quantification of estrogen responsive genes, *E2F1*, *BCL2* messenger RNA levels in the human breast tumor cell line, MCF7 treated with the indicated compounds (10 μM, 48 h) (n = 3 separate experiments per group). *GAPDH* served as internal loading control for normalization.

(E) Trypan blue exclusion cell viability assessment conducted in SH-SY5Y cells treated with increasing concentrations of estriol or SG13-136 (0, 10, 50, 100, 200, 500 μM, 48 h) (n = 6 separate experiments per group).

(F) Chemical structure of biotin-conjugated SG13-136 (also named SG13-158), along with chemical formula and mass.

(G) Amyloid-like aggregation of recombinant tau incubated for three days (20 μM in PBS with 5 μM heparin) in the presence of indicated compounds (50 μM), monitored by thioflavin T fluorescence assay (n = 3 per group). Thioflavin T measurement of recombinant tau at day 0 served as baseline, with DMSO as vehicle control.

Data in all panels represent mean ± standard error of the mean. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, determined by one-way (A, B, C, D, and E) or two-way (G) analysis of variance (ANOVA) followed by Tukey's post hoc analysis.


A
CTRL
Estriol
SG13-136
Body weight (g)
(Day)
B
Latency to descend the pole (s)
ns
**
*
DMSO
Estriol
SG13-136
C
Latency to fall off rotarod (s)
ns
**
**
DMSO
Estriol
SG13-136
D
Relative mRNA levels (Ebag9)
ns
*
*
DMSO
Estriol
SG13-136
E
Relative mRNA levels (Ccnd1)
ns
**
*
DMSO
Estriol
SG13-136
F
Relative mRNA levels (Ebag9)
ns
DMSO
SG13-136
G
Relative mRNA levels (Ccnd1)
ns
DMSO
SG13-136
H
Enlarged images
DMSO
SG13-136
DMSO
SG13-136
Heart
Liver
Kidney
Lung
Spleen
50μm
12.5μm
I
Non-injection
AAV-eGFP +PBS
AAV-αSyn +αSyn PFF
TH
GFP
Merged
100μm
J
Non-injection
AAV-eGFP +PBS
AAV-αSyn +αSyn PFF
TH
αSyn
Merged
100μm
K
Striatum (STR)
AAV-eGFP +PBS
AAV-αSyn +αSyn PFF
TH
Vehicle
SG13-136
500μm
L
Optical density (% of control)
***
***
SG13-136
-
+
-
+
AAV-eGFP +PBS
AAV-αSyn +αSyn PFF

Supplementary Figure 2. Related to Figure 5. The steroid derivative, SG13-136, devoid of estrogenic activity, showed no organ toxicity and conferred protection to dopaminergic axon in a αSyn PFF/AAV-αSyn injection PD mouse model.

(A) Measurement of mouse body weights (every two days) with daily administrations of vehicle, estriol, or SG13-136 (0.5 mg/kg/d, p.o. for 20 days) (n = 4 mice per group).

(B) Assessment of bradykinesia in wild-type mice administered with vehicle DMSO, estriol, or SG13-136 (0.5 mg/kg, p.o. once daily for three weeks) or vehicle monitored by pole test (n = 4 mice per group).

(C) Assessment of motor coordination in wild-type mice administered with vehicle DMSO, estriol, or SG13-136 (0.5 mg/kg, p.o. once daily for three weeks) or vehicle monitored by pole test (n = 4 mice per group).

(D) Quantification of the estrogen responsive gene, *Ebag9* messenger RNA levels in brains from the mice with the indicated compound administration (0.5 mg/kg/d estriol, or SG13-136, p.o., for three weeks) monitored by real-time quantitative PCR and normalized by *Gapdh* internal loading control (n = 4 mice per group).

(E) Quantification of the estrogen responsive gene, *Ccnd1* messenger RNA levels in brains from the mice with the indicated compound administration (0.5 mg/kg/d estriol, or SG13-136, p.o., for three weeks) monitored by real-time quantitative PCR and normalized by *Gapdh* internal loading control (n = 4 mice per group).

(F, G) Quantification of the estrogen responsive genes, *Ebag9*, *Ccnd1* messenger RNA levels in liver tissues from the mice with the administration of SG13-136 (0.5 mg/kg/d, p.o., for three weeks) monitored by real-time quantitative PCR and normalized by *Gapdh* internal loading control (n = 4 mice per group).

(H) Representative H&E staining images of tissue sections (heart, liver, kidney, lung, and spleen) from the mice administered with vehicle or SG13-136 (0.5 mg/kg/d, p.o., for three weeks). Enlarged images were also presented in the right panel. Scale bar = 50, and 12.5 μm, respectively.

(I, J) Representative co-immunofluorescence images showing the expression and distribution of eGFP and α-synuclein in dopaminergic neurons labeled with TH on coronal ventral midbrain sections from each experimental mouse groups. Scale bar = 100 μm.

(K) Representative anti-TH immunohistochemistry images of the striatum coronal sections from each mouse group with the indicated nigral injections and SG13-136 p.o. administration. Scale bar = 500 μm.

(L) Quantification of relative TH-stained dopaminergic axon terminal densities in the striatum of the indicated experimental groups (n = 5 mice per group).

Data in all panels represent mean ± standard error of the mean. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, determined by unpaired two-tailed Student's t-test (F, and G), one-way (B, C, D, and E) or two-way (L) analysis of variance (ANOVA) followed by Tukey's post hoc analysis. n.s., non-significant.