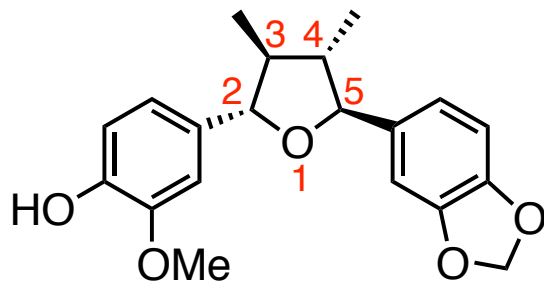


Talaumidinの誘導体合成 と神経栄養因子様活性

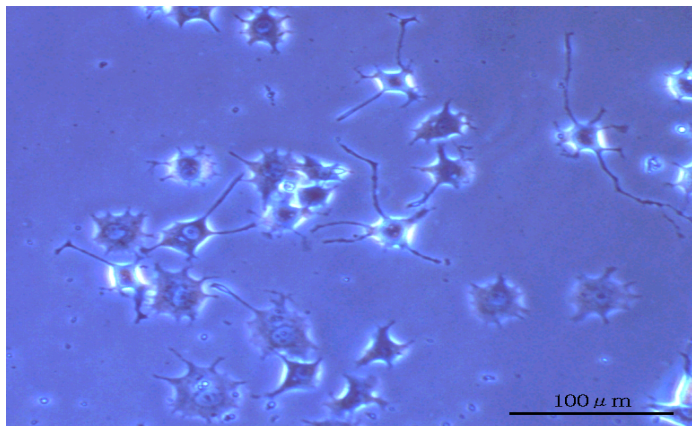
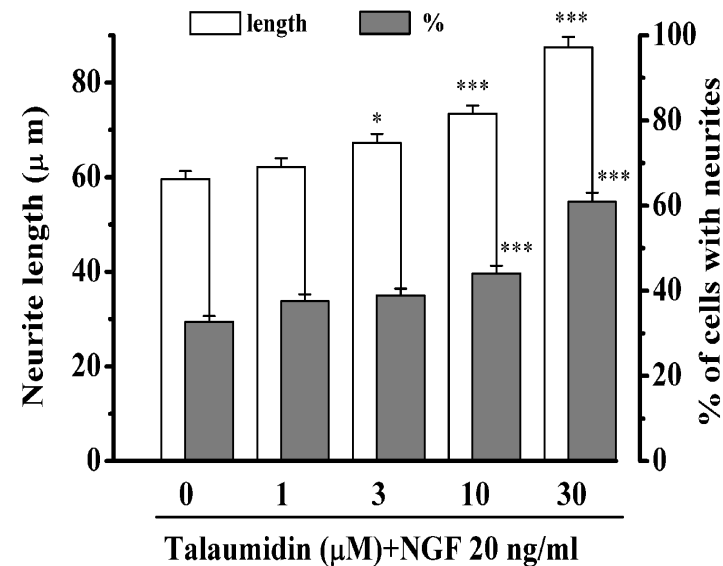
徳島文理大薬

○座波克圭、今井顕子、原田研一、久保美和
松井敦聡、山田恵子、赤木正明、福山愛保

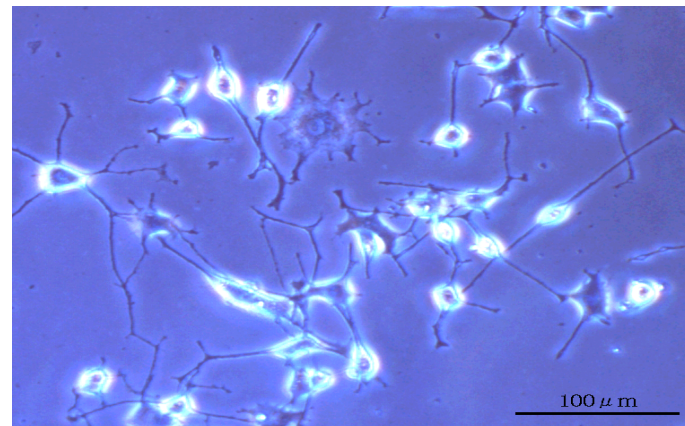
Talaumidin promotes neurite outgrowth in NGF-differentiating PC12 cells



Talaumidin (**1**)
isolated from Brazilian plant
Aristolochia arcuata



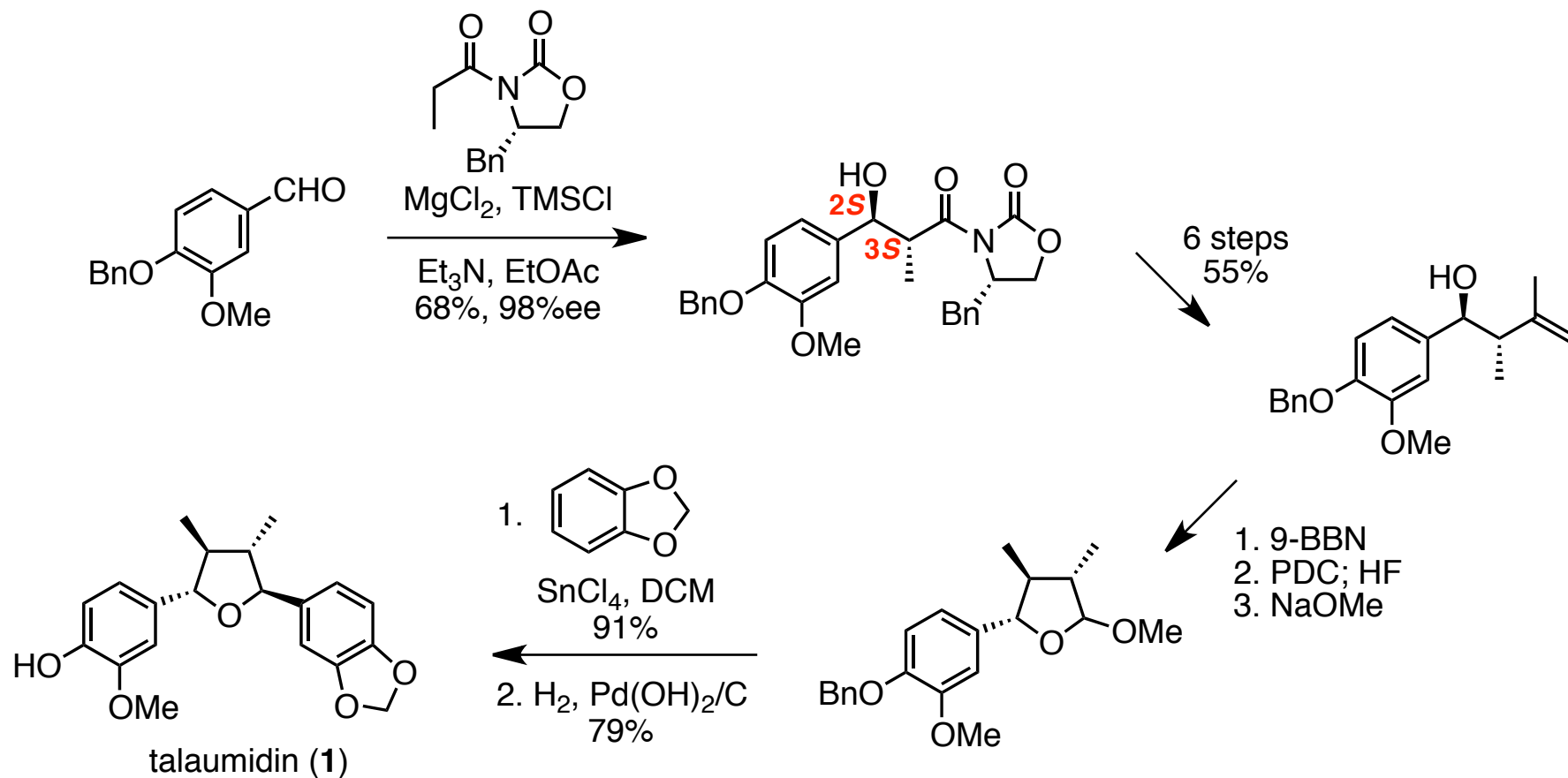
control (NGF 20 ng/mL)



1: 30 μM + NGF 20 ng/mL

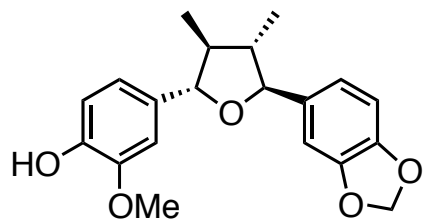
1. H. Zhai, M. Nakatsukasa, Y. Mitsumoto, Y. Fukuyama, *Planta Med.*, **70**, 598-602 (2004).

Total synthesis of (-)-talaumidin¹

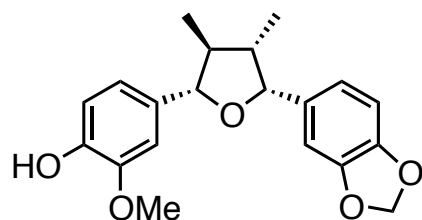


1. T. Esumii, D. Hojyo, H. Zhai, Y. Fukuyama, *Tetrahedron Lett.*, **47**, 3979-3983 (2006).
Synthesis of **1** by other groups: C. E. Rye, D. Barker, *Synlett*, **20**, 3315-3319 (2009).
H. Kim, C. M. Wooten, Y. Park, J. Hong, *Org. Lett.*, **9**, 3965-3968 (2007).
K. Matcha, S. Ghosh, *Tetrahedron Lett.*, **49**, 3433-3436 (2008)

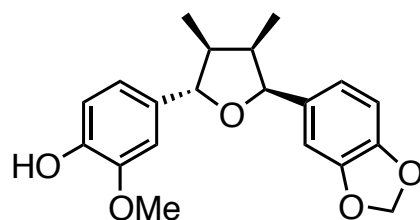
(-)-Talaumidin and seven stereoisomers



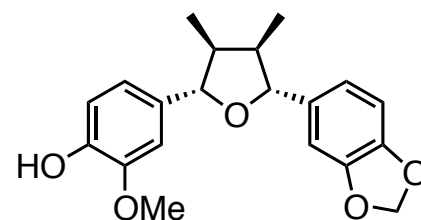
(2S,3S,4S,5S)-talaumidin



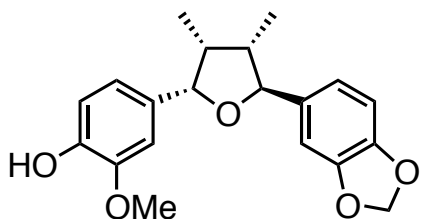
(2S,3S,4S,5R)-**1a**¹



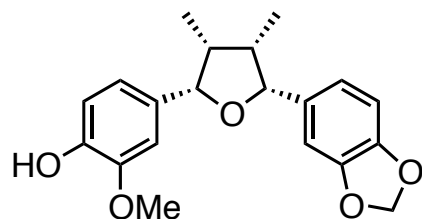
(2S,3S,4R,5S)-**1b**¹



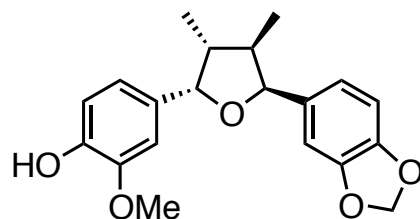
(2S,3S,4R,5R)-**1c**²



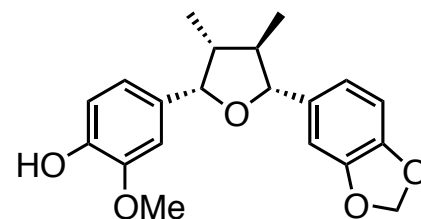
(2S,3R,4S,5S)-**1d**³



(2S,3R,4S,5R)-**1e**



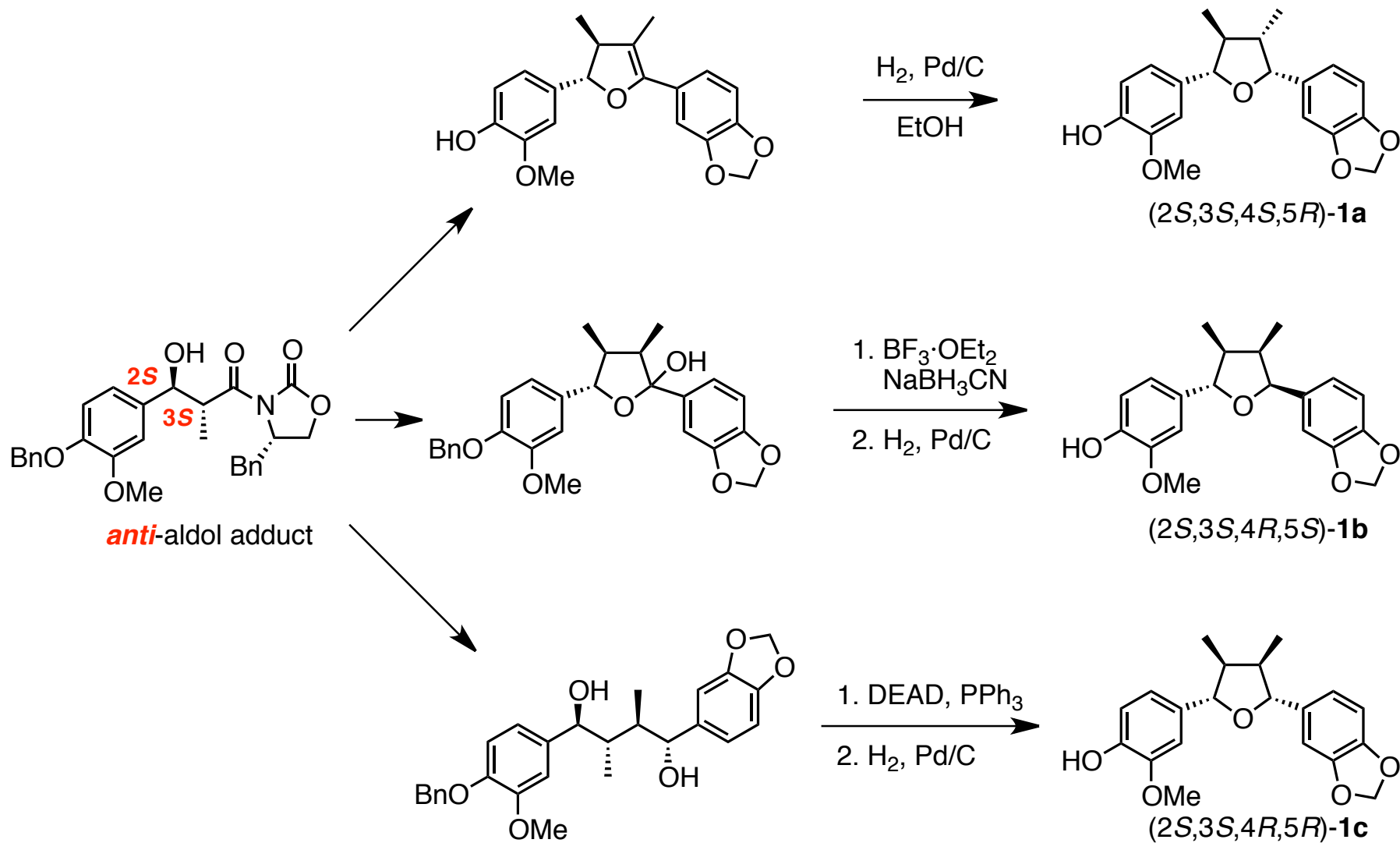
(2S,3R,4R,5S)-**1f**



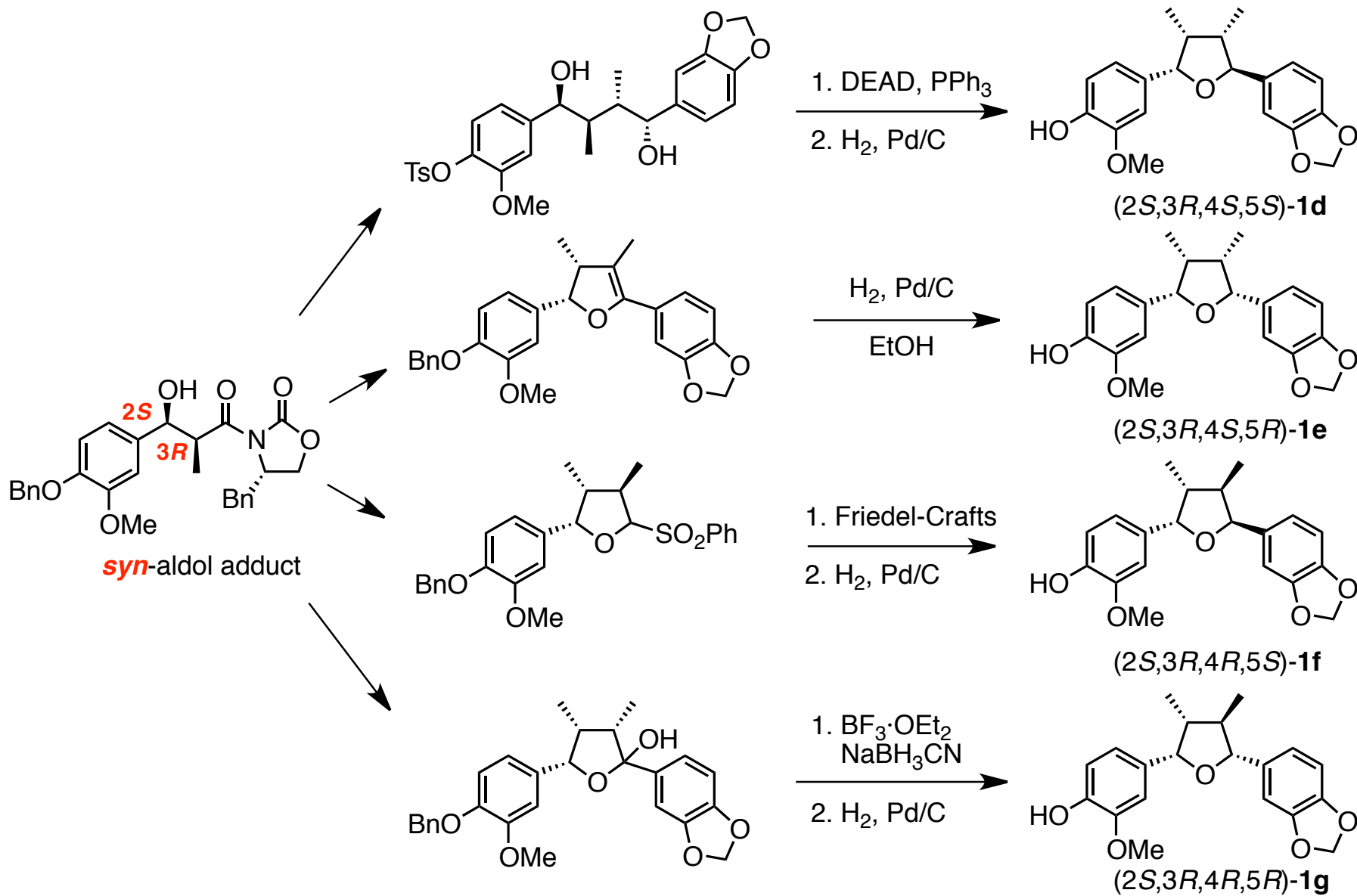
(2S,3R,4R,5R)-**1g**

1. Y. Fukuyama, K. Harada, T. Esumi, D. Hojyo, Y. Kujime, N. Kubo, M. Kubo, H. Hioki, *Heterocycles*, **76**, 551-567 (2008).
2. K. Harada, N. Kubo, K. Tanabe, M. Kubo, T. Esumi, H. Hioki, Y. Fukuyama, *Heterocycles*, **82**, 1127-1132 (2011).
3. K. Harada, H. Horiuchi, K. Tanabe, Rich G. Carter, T. Esumi, M. Kubo, H. Hioki, Y. Fukuyama, *Tetrahedron Lett.*, **52**, 3005-3008 (2011).

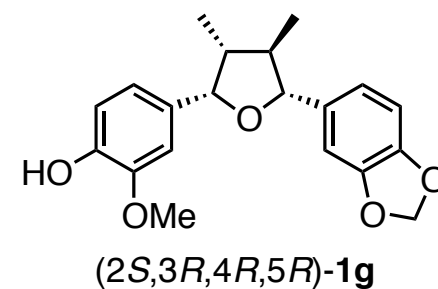
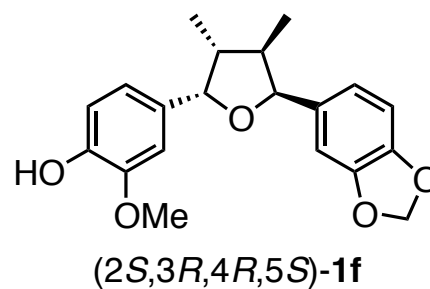
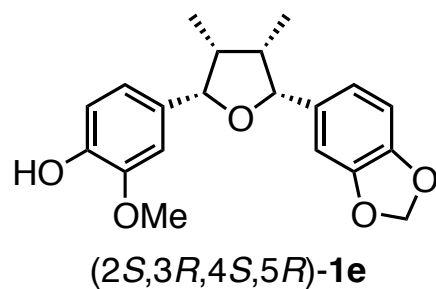
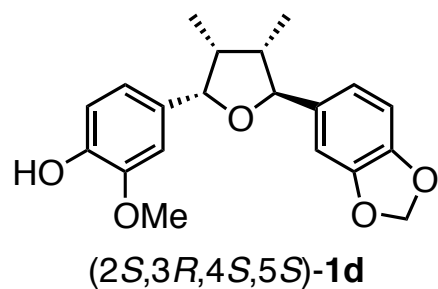
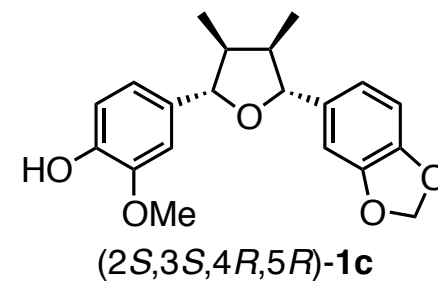
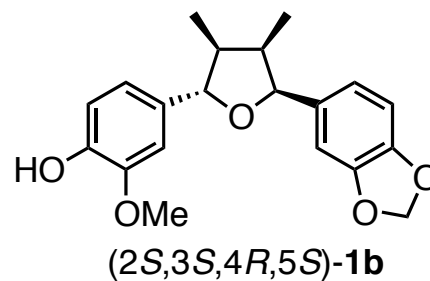
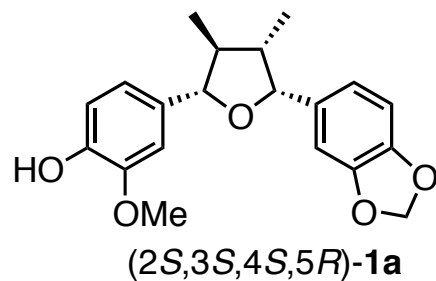
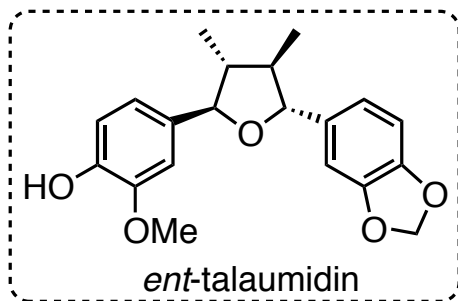
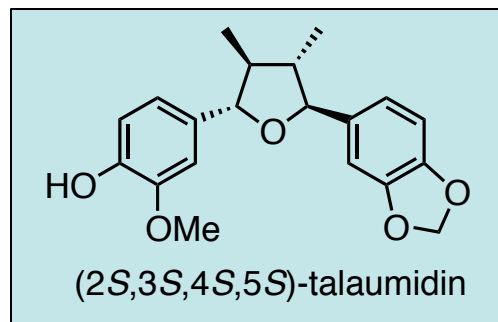
Synthesis of isomers 1a-1c having 2S,3S configuration



Synthesis of isomers 1d-1g having 2*S*,3*R* configuration

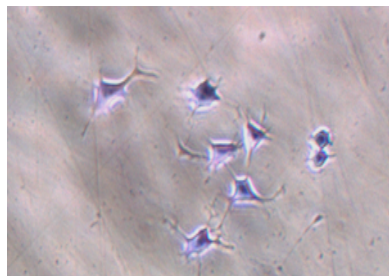


Talaumidin and its stereoisomers

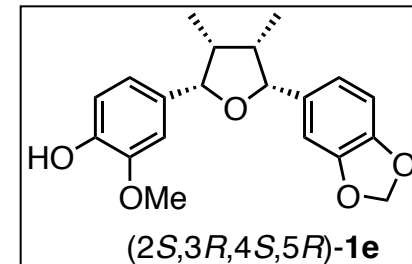
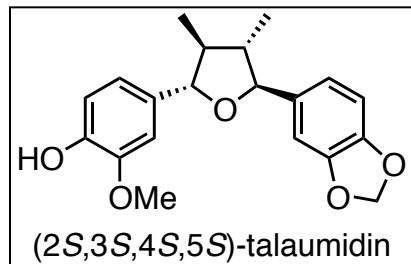
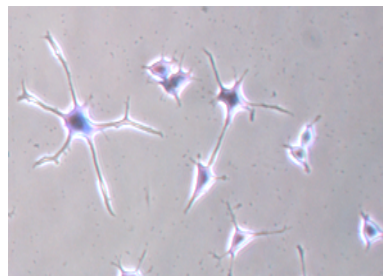


Neurite-outgrowth promoting activity in NGF differentiating PC12 cells

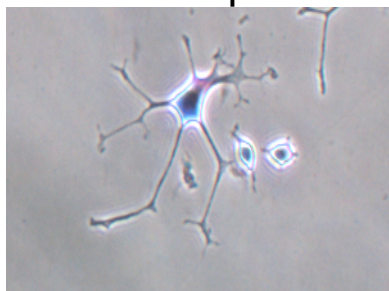
NGF
2 ng/mL



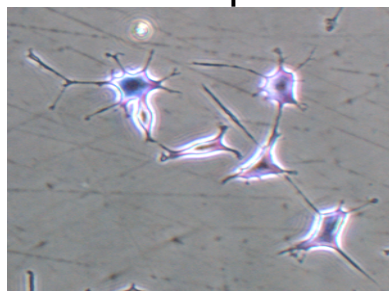
NGF 2 ng/mL+
Talaumidin 30 μM



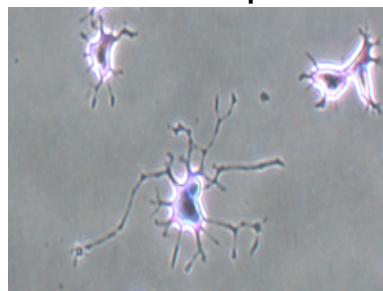
NGF 2 ng/mL+
1a 30 μM



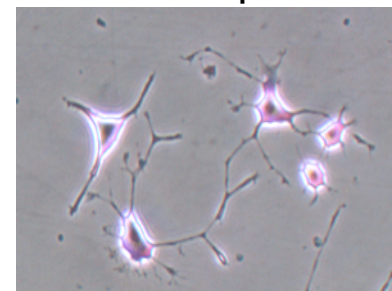
NGF 2 ng/mL+
1b 30 μM



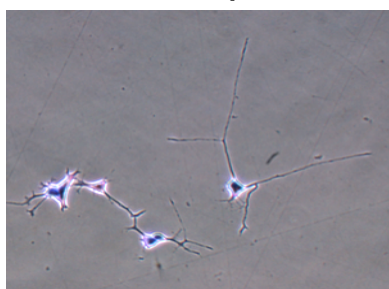
NGF 2 ng/mL+
1c 30 μM



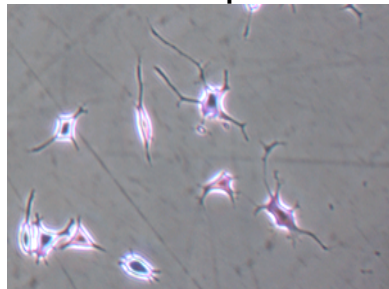
NGF 2 ng/mL+
1d 30 μM



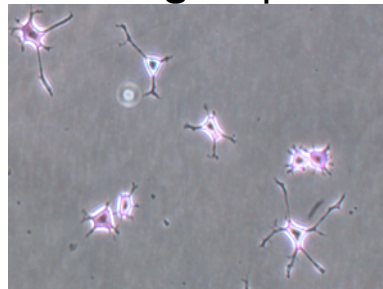
NGF 2 ng/mL+
1e 30 μM



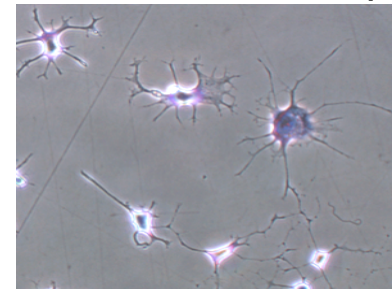
NGF 2 ng/mL+
1f 30 μM



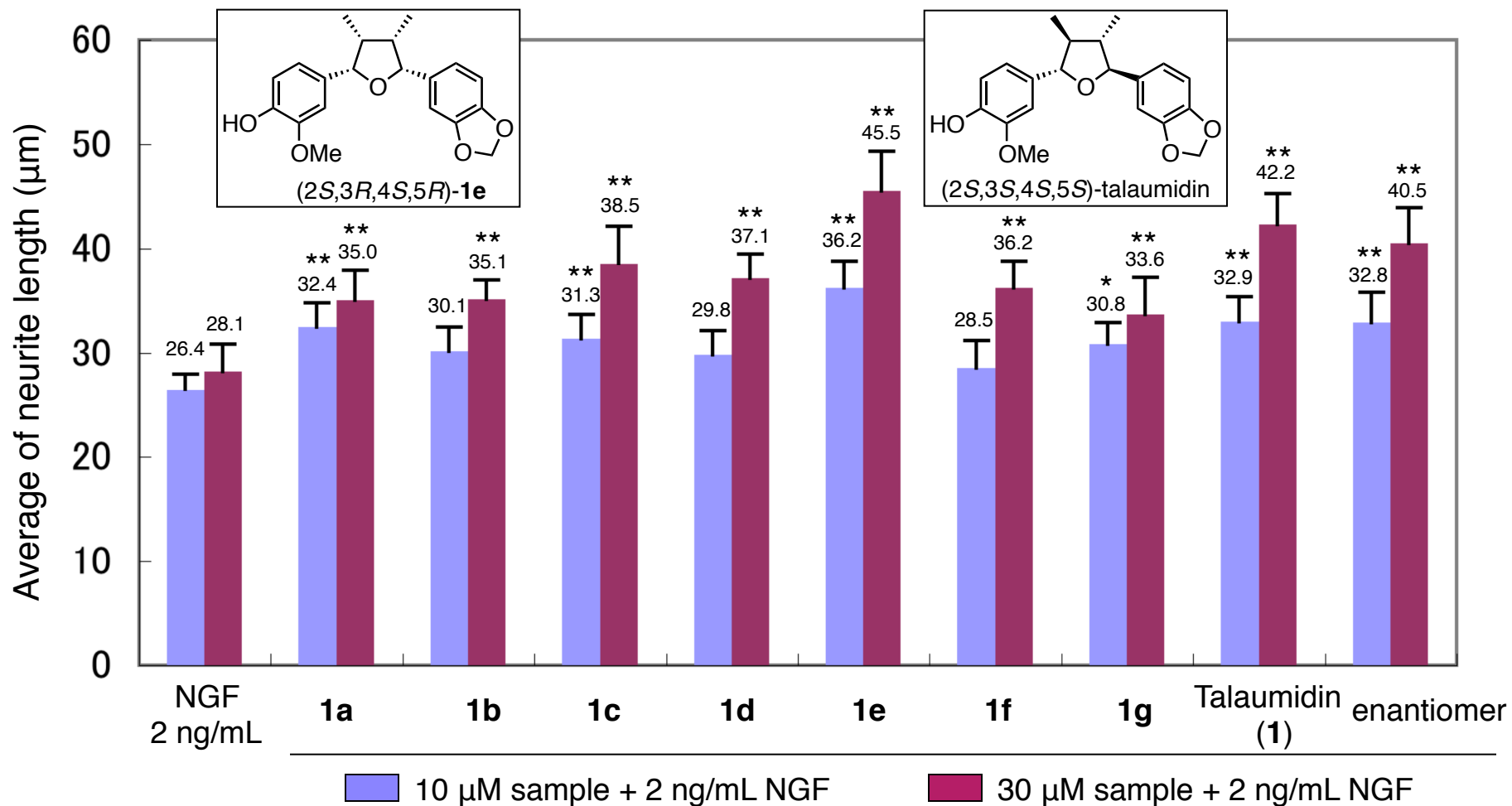
NGF 2 ng/mL+
1g 30 μM



NGF 2 ng/mL+
ent-Talaumidin 30 μM

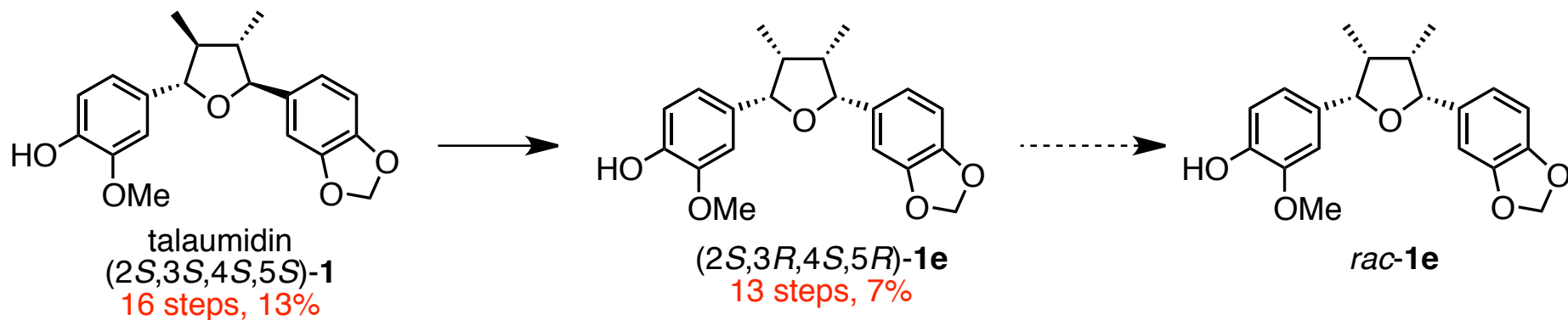


Comparison of neurite length of NGF-differentiating PC12 cells promoted by talaumidin and its stereoisomers at 10 and 30 μM

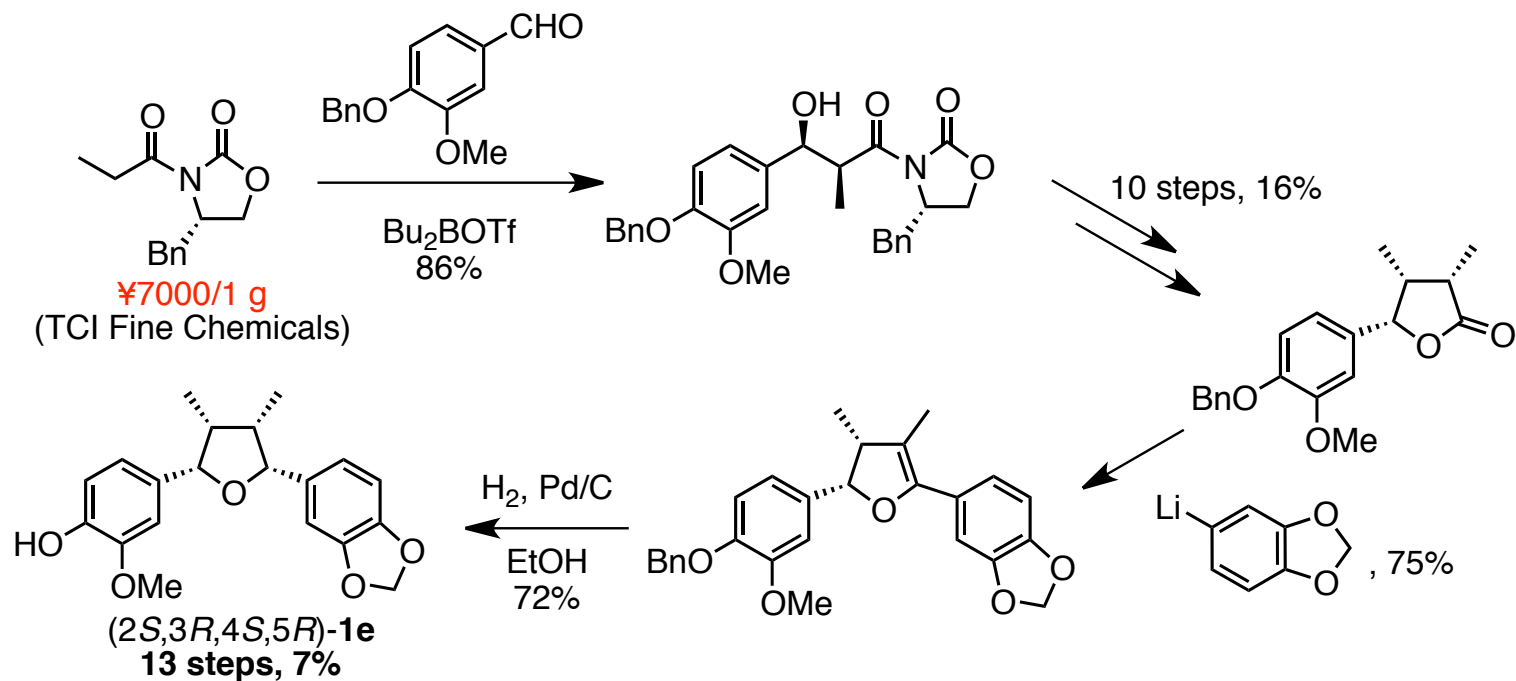


PC12 cells were cultured in 24-well plate in DMEM/10%HS+5%FBS for 1day at the density of 2000 cells/ cm^2 then medium change to DMEM/2%HS+ 1%FBS with control (0.5% EtOH), NGF 2 ng/mL, NGF 2 ng/mL + samples 10mM and 30mM. after 96 hr, PC12 cells were fixed and stained with methylene blue, then were quantified for the neurite length. At least 100 cells were selected for calculating the neurite length. Data were expressed a mean as \pm SE. *, $P < 0.05$; **, $P < 0.01$ compared with NGF by Dunnett's t-test.

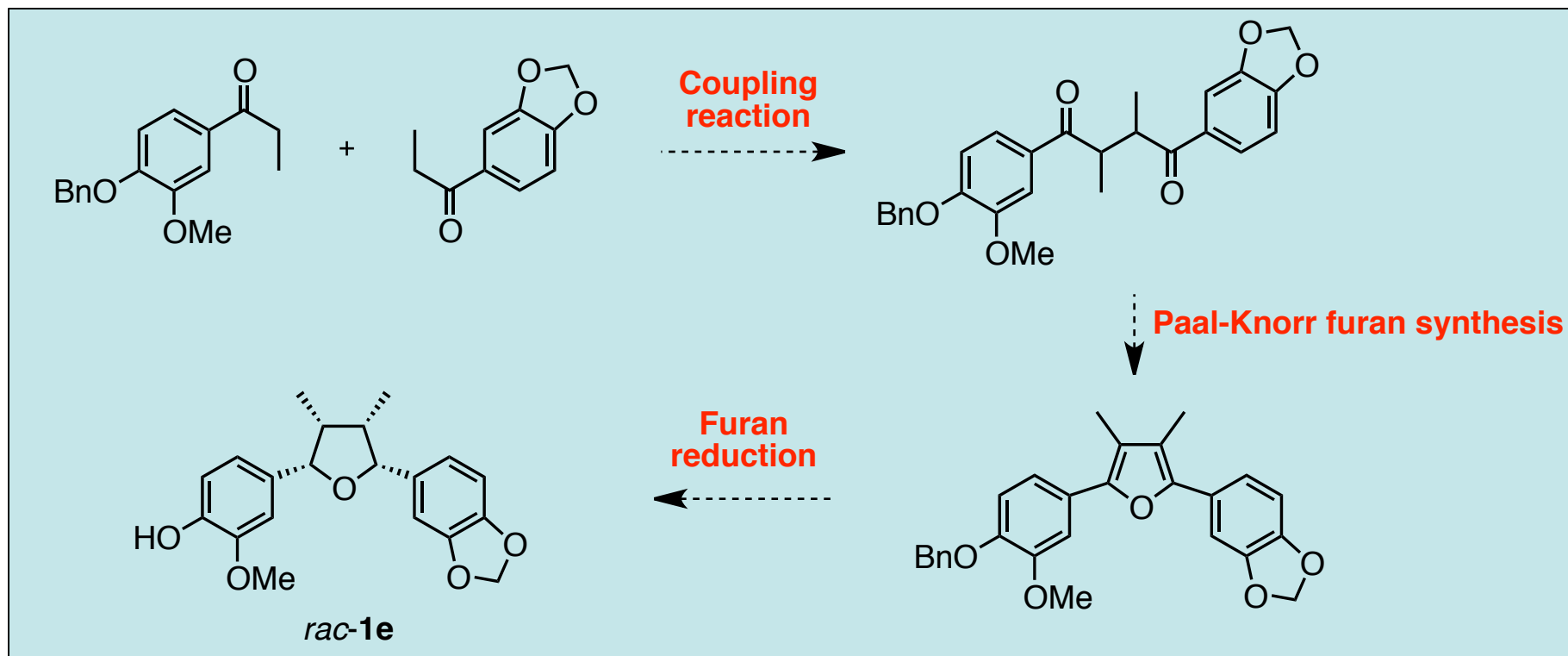
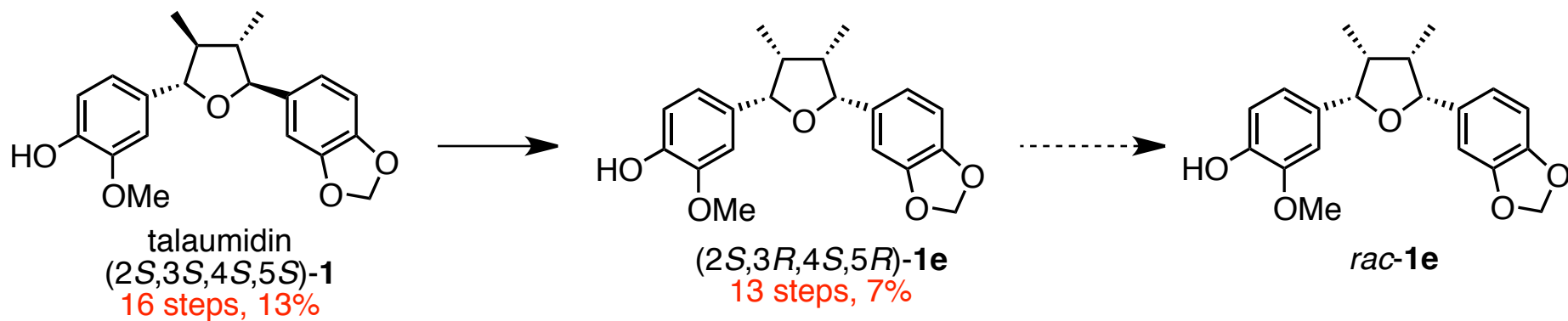
Derivatization of talaumidin



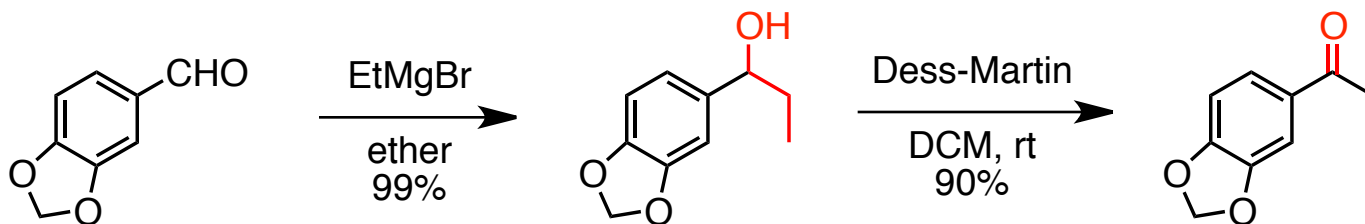
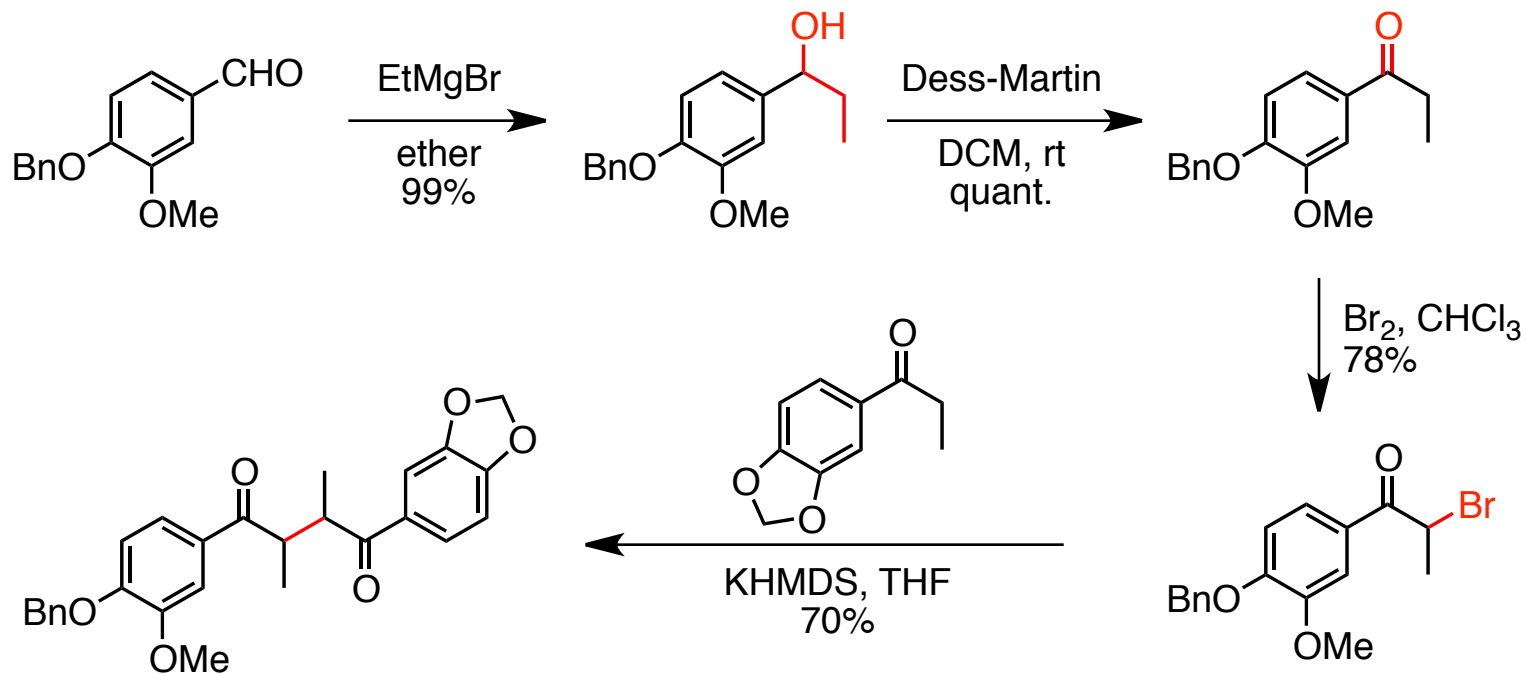
Synthesis of (2*S*,3*R*,4*S*,5*R*)-1e



Synthetic plan of rac-1e



Preparation of 1,4-diketone



Wako pure chemical product
¥1880 / 25 g

Wako pure chemical product
¥19650 / 5 g

Completion of the synthesis of *rac-1e*

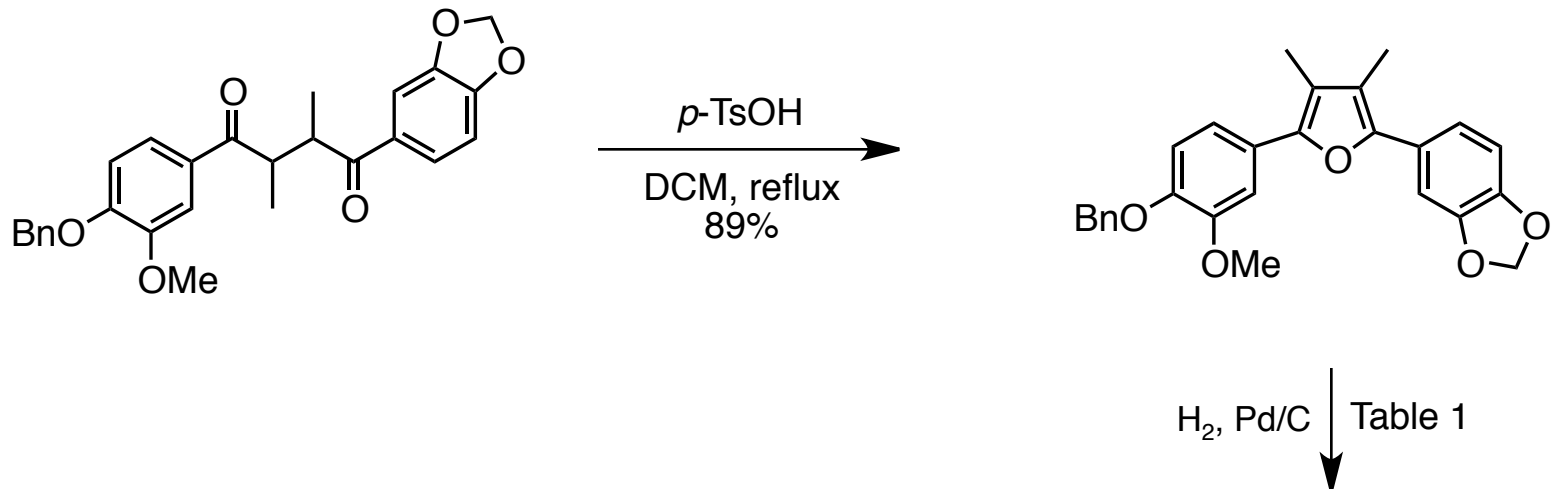
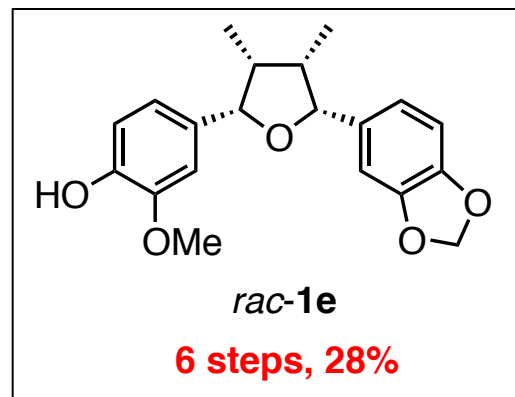


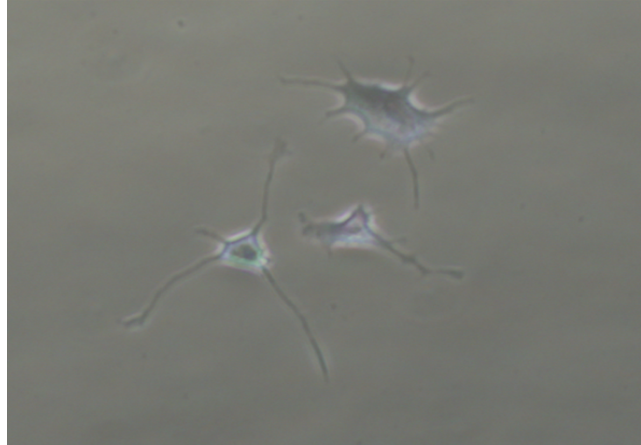
Table 1

entry	solvent	conc.	time	yield
1	AcOH/MeOH =1:1*	0.1 M	24 h	29%
2	AcOH/MeOH =1:1	0.05 M	24 h	25%
3	EtOH	0.1 M	24 h	36%
4	EtOH	0.005 M	96 h	68%

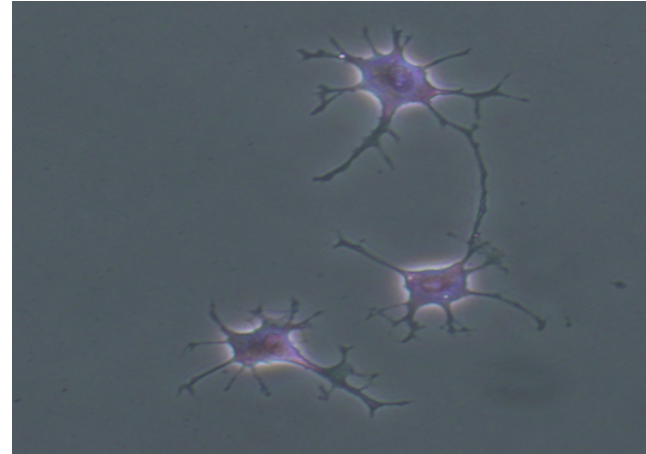


*Blears, J. G.; Haworth, R. D. *J. Chem. Soc.*, 1985-1987 (1958).

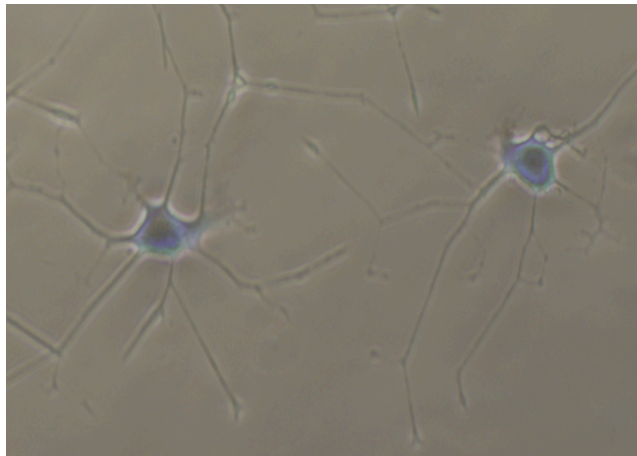
Neurite-outgrowth promoting activity in NGF differentiating PC12 cells



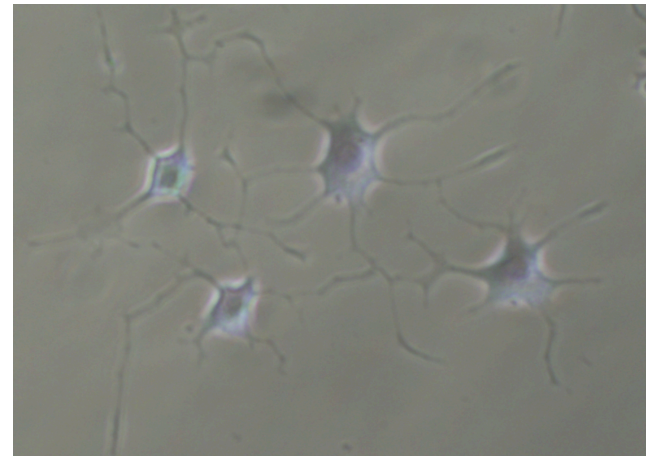
NGF 2 ng/mL



Talaumidin 10 μ M + NGF 2 ng/mL

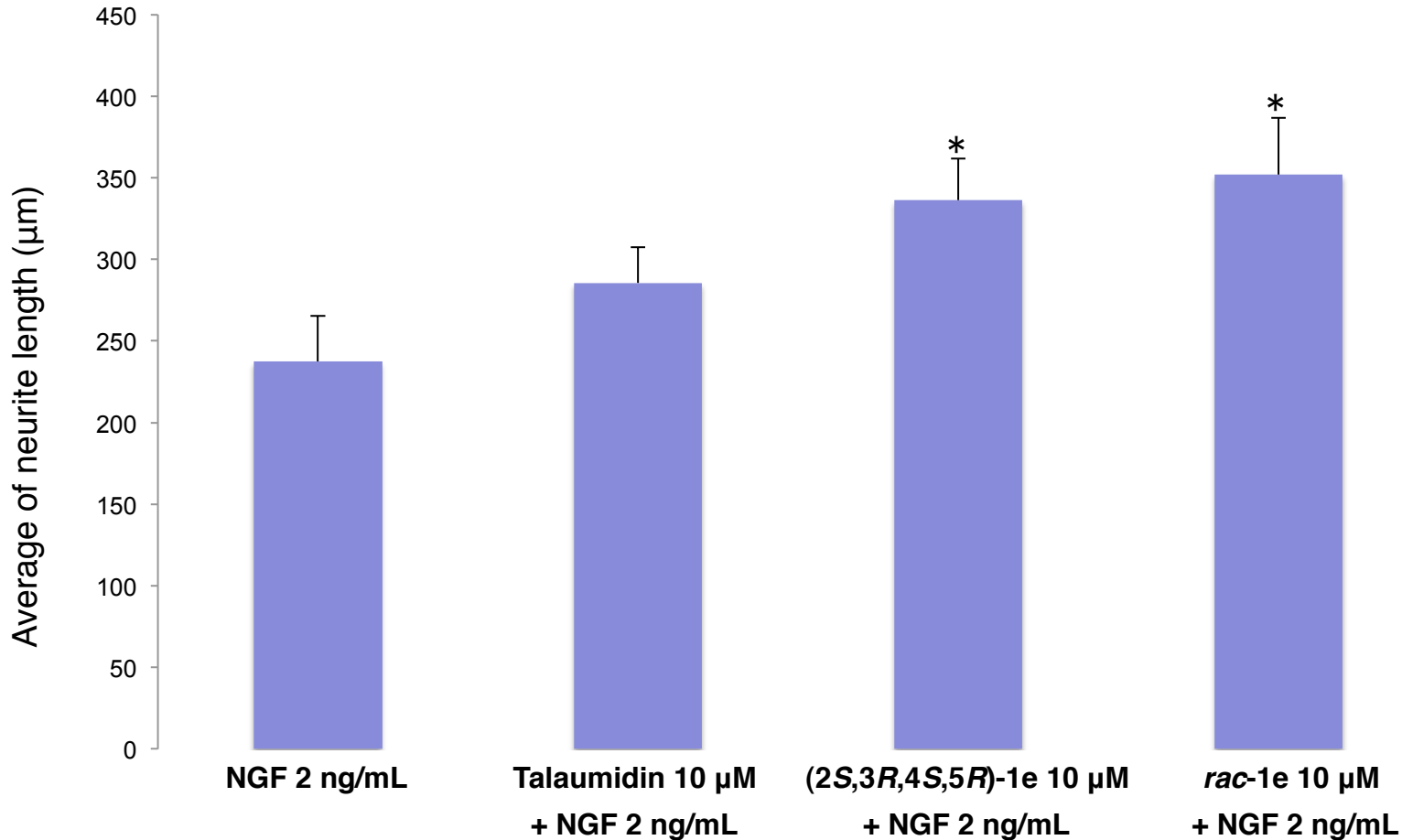


(2*S*,3*R*,4*S*,5*R*)-1e 10 μ M + NGF 2 ng/mL



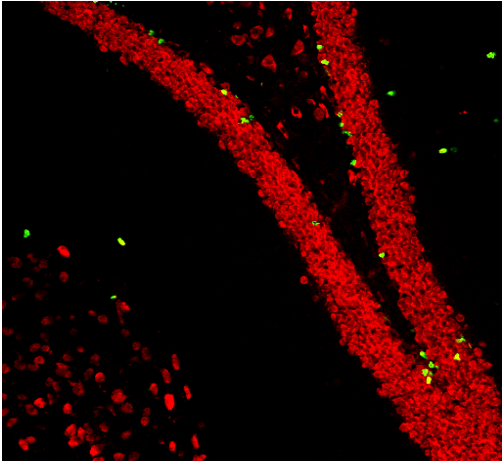
rac-1e 10 μ M + NGF 2 ng/mL

Comparison of neurite length of NGF-differentiating PC12 cells promoted by talaumidin, (2*S*,3*R*,4*S*,5*R*)-1e and *rac*-1e

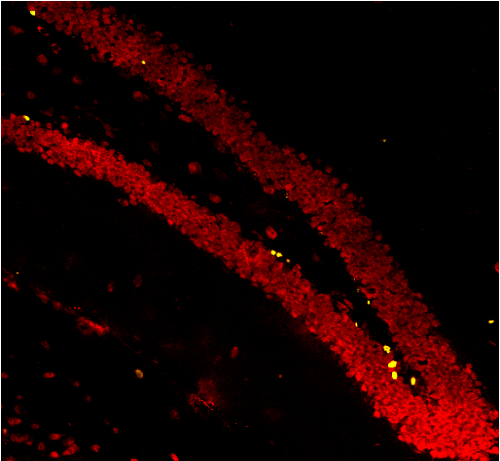


PC12 cells were cultured in 24-well plate in DMEM/10%HS+5%FBS for 1day at the density of 2000 cells/cm² then medium change to DMEM/2%HS+ 1%FBS with control (0.5% EtOH), NGF 2 ng/mL, NGF 2 ng/mL + samples 10 mM. after 96 hr, PC12 cells were fixed and stained with methylene blue, then were quantified for the neurite length. At least 100 cells were selected for calculating the neurite length. Data were expressed a mean as \pm SE. *, $P < 0.05$; compared with NGF by Dunnett's t-test.

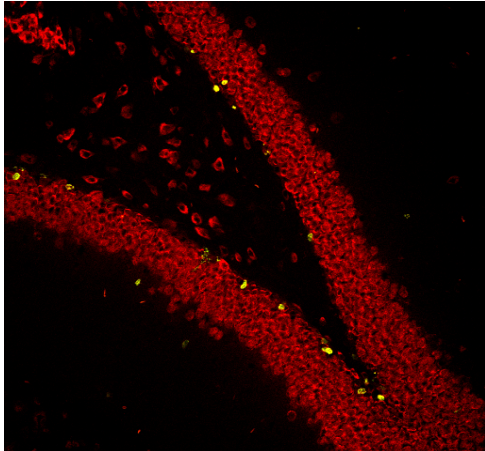
Hippocampal neurogenesis in olfactory bulbectomized (OBX) mice



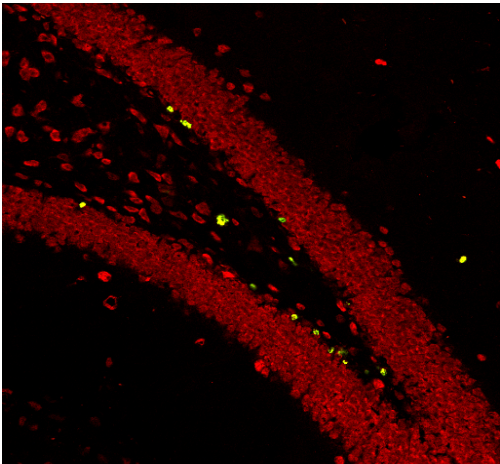
normal



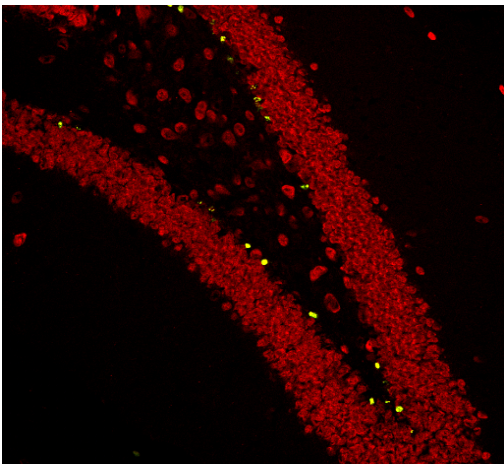
OBX



OBX-vehicle



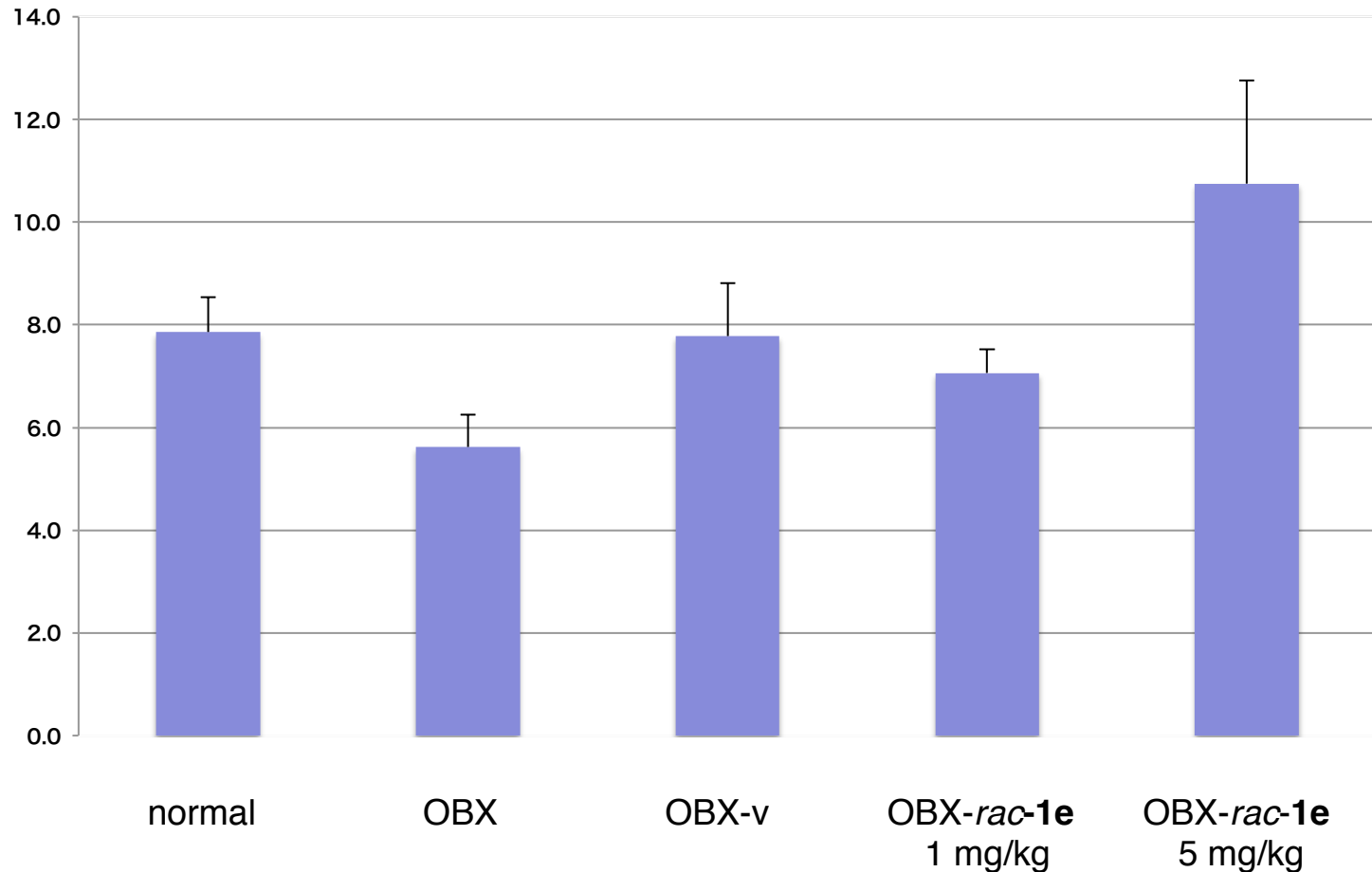
OBX-*rac-1e*
1 mg/kg



OBX-*rac-1e*
5 mg/kg

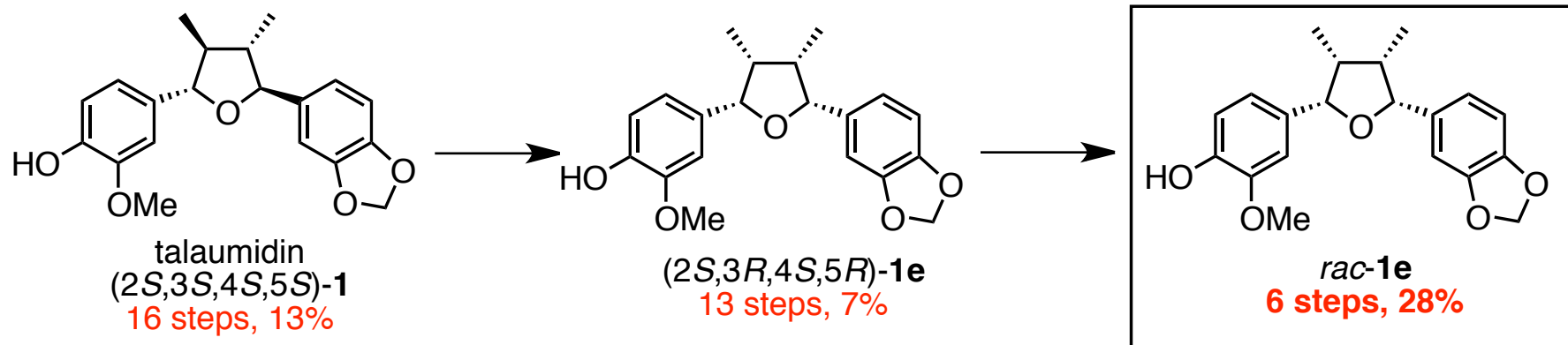
red: NeuN
green: BrdU

Hippocampal neurogenesis in olfactory bulbectomized (OBX) mice



To determine the rate of hippocampal neurogenesis, 5-bromo-2-deoxyuridine (BrdU) (50 mg/kg) was administered by i. p. once a day for 7 days for 22 to 28 days after OBX operation. On day 30, mice were sacrificed. The number of BrdU/NeuN double-positive cells in the dentate gyrus of hippocampus was counted.

Conclusion



Neurite outgrowth promoting activity in PC12 cells

$rac-1e \cong (2S,3R,4S,5R)-1e > \text{talaumidin}$