Alkoxy-auxins are selective inhibitors of auxin transport mediated by PIN, ABCB and AUX1 transporters

Authors

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Supplemental data (Supplemental Figures S1 to S7.)

SI-Reference

Nagashima A, Suzuki G, Uehara Y, Saji K, Furukawa T, Koshiba T, Sekimoto M, Fujioka S, Kuroha T, Kojima M, Sakakibara H, Fujisawa N, Okada K, Sakai T (2008) Phytochromes and cryptochromes regulate the differential growth of Arabidopsis hypocotyls in both a PGP19-dependent and a PGP19-independent manner. Plant Journal **53:** 516-529

S1

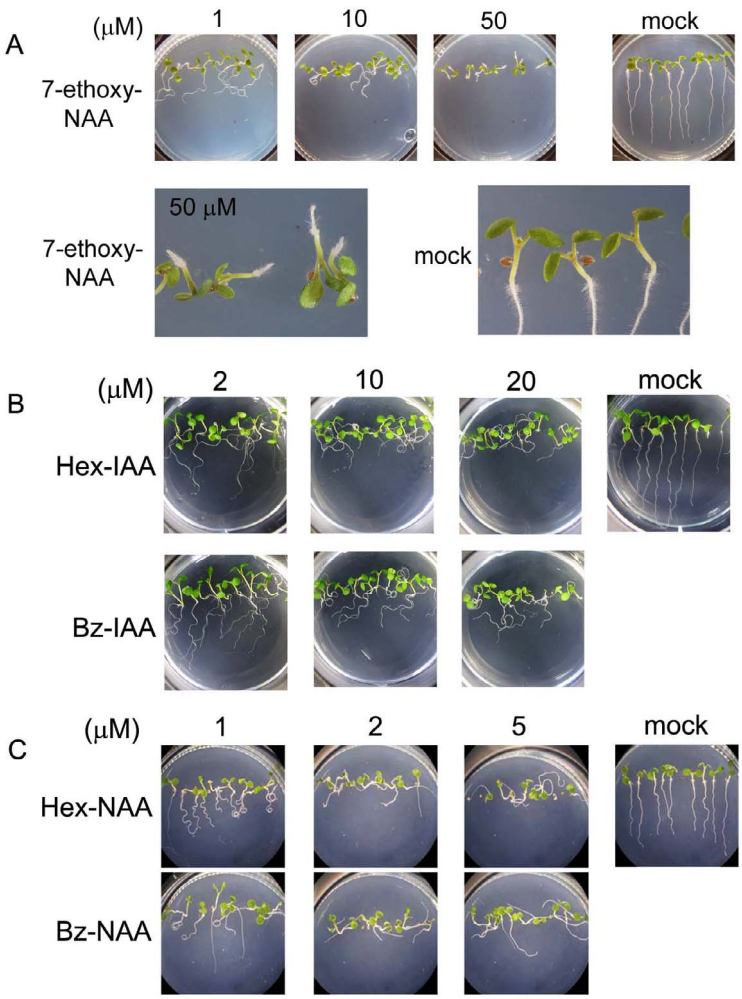


Fig. S1 Effects of alkoxy-auxins on the growth of *Arabidopsis* plants. *Arabidopsis* seedlings were grown vertically on the germination (GM) medium containing 1.5% sucrose and 1.4% agar supplemented with alkoxy-auxin at indicated concentration. (A) Effects of 7-ethoxy-NAA on seedling growth. 7-Ethoxy-NAA at 1 μM inhibited the root grapitropism. The seedling treated with 50 μM 7-ethoxy-NAA showed similar phenotype to auxin treated plant, such as root inhibition and root hair promotion. This suggests that 7-ethoxy-NAA has weak auxin activity. (B) Inhibitory effects of 5-hexyloxy-IAA, Hex-IAA (**6a**) and 5-benzyloxy-IAA, Bz-IAA (**7a**) on seedling growth. The seedlings were grown for 6 days. Hex-IAA and Bz-IAA showed potent inhibition of root gravitropism. (C) Inhibitory effects of 5-hexyloxy-NAA, Hex-NAA (**6b**) and 5-benzyloxy-IAA, Bz-NAA (**7b**) on seedling growth. The seedlings were grown for 5 days. Hex-NAA and Bz-NAA showed potent inhibition of root gravitropism.

S2

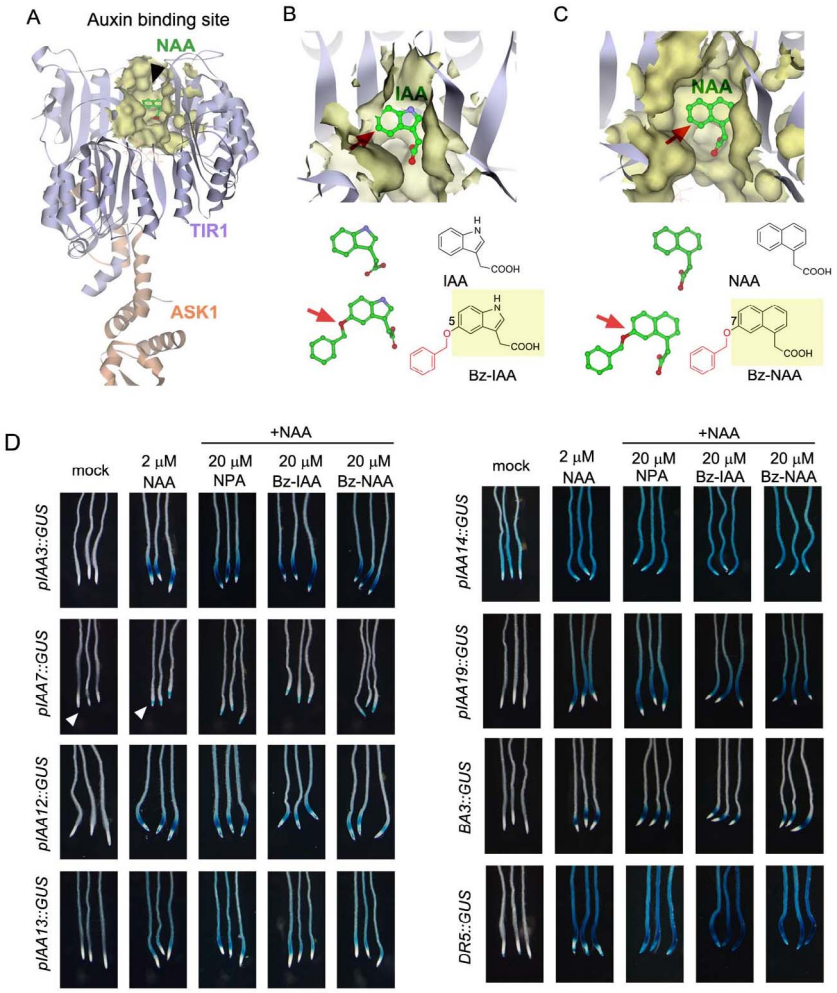


Fig. S2. Effects of alkoxy-auxin on SCF ^{TIR1} auxin signaling pathway.
(A-C) Crystal structure of auxin - TIR1 receptor complex and the chemical structure of IAA, NAA and Bz-IAA and Bz-NAA. (A) the crystal structures of ASK1-TIR1-NAA complex (PDB ID: 2P1O) Black arrow indicate the auxin binding site of TIR1. The protein surface of auxin binding site was calculated and displayed as yellow colored. (B) The crystal structure of IAA in auxin binding site of TIR1 (PDB ID: 2P1Q) and chemical structure of IAA and Bz-IAA. (C) The crystal structure of NAA in auxin binding site of TIR1 (PDB ID: 2P1O). The chemical structure of NAA and Bz-NAA. Red arrows indicate the substitution position of benzyloxy group (IAA at C-5 and NAA at C-7 position).
(D) Effects of Bz-IAA (**7a**) and Bz-NAA (**7b**) on auxin-responsive promoter activities regulated by SCF ^{TIR1} auxin signaling pathway. *Arabidopsis* reporter lines, *pIAA3*, *pIAA7*, *pIAA12*, *pIAA13*, *pIAA14* and *pIAA19::GUS* contains native *Arabidopsis Aux/IAA* promoter::GUS reporter gene. The *BA3::GUS* and *DR5::GUS* lines have synthetic auxin-responsive composite promoter derived from Pea Aux/IAA and Soybean GH3 gene. The 5d-old seedlings were incubated with the chemicals in the liquid GM medium for 10 h.

S3

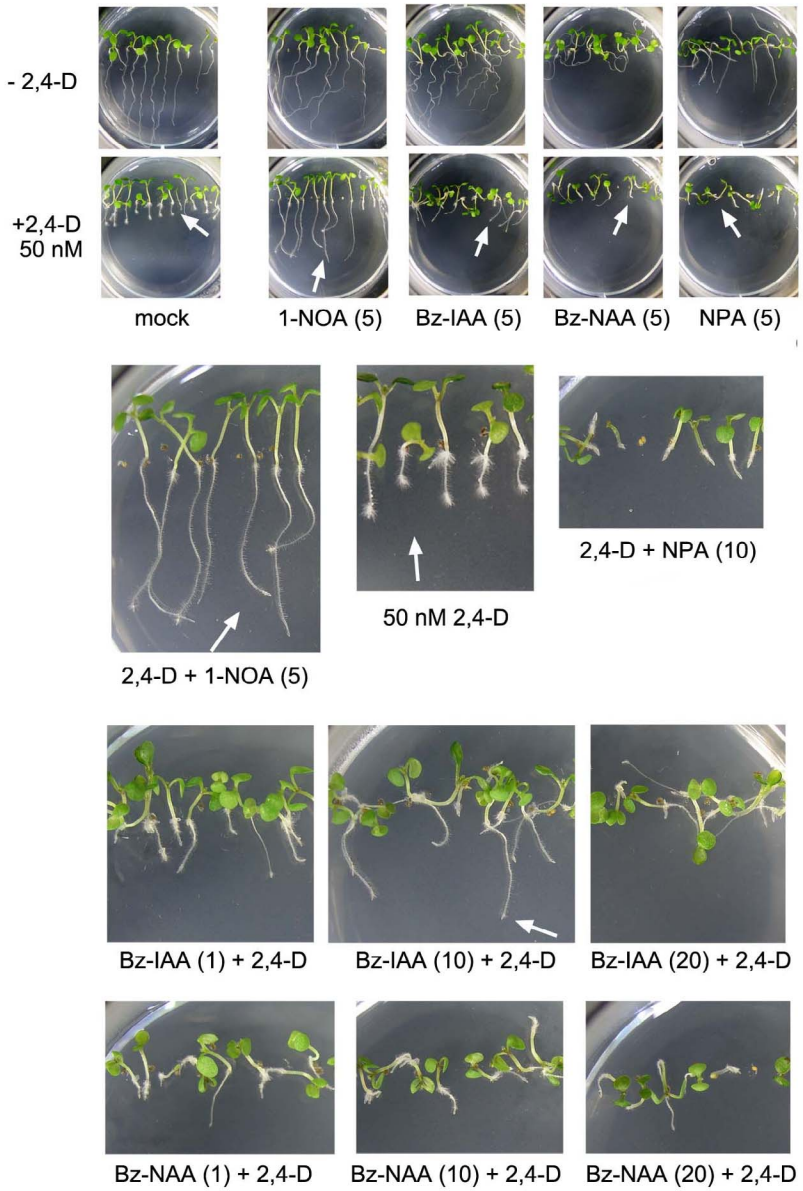


Fig. S3. The auxin influx inhibitor 1-naphthyloxy acetic acid (1-NOA) and Bz-IAA, but not Bz-NAA restored 2,4-D-inhibited root growth.

Arabidopsis seedling was grown vertically on GM agar medium containing 1.5 % sucrose and 1.4 % agar supplemented with inhibitors in the presence or absence of 50 nM of synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D). The values in the parenthesis present the concentration of chemicals at μM. 2,4-D treatment inhibited the primary root growth and promoted root heir formation as indicated by arrows. 1-NOA at 5 μM dramatically rescued 2,4-D-induced root phenotypes by blocking the 2,4-D uptake. Bz-IAA at 10 μM significantly recovered from 2,4-D-induced root phenotypes by reducing 2,4-D uptake. In contrast, Bz-NAA and NPA at 10 μM enhanced the primary root inhibition by 2,4-D. This effect might be due to the accumulation of 2,4-D by the inhibition of 2,4-D efflux transport.

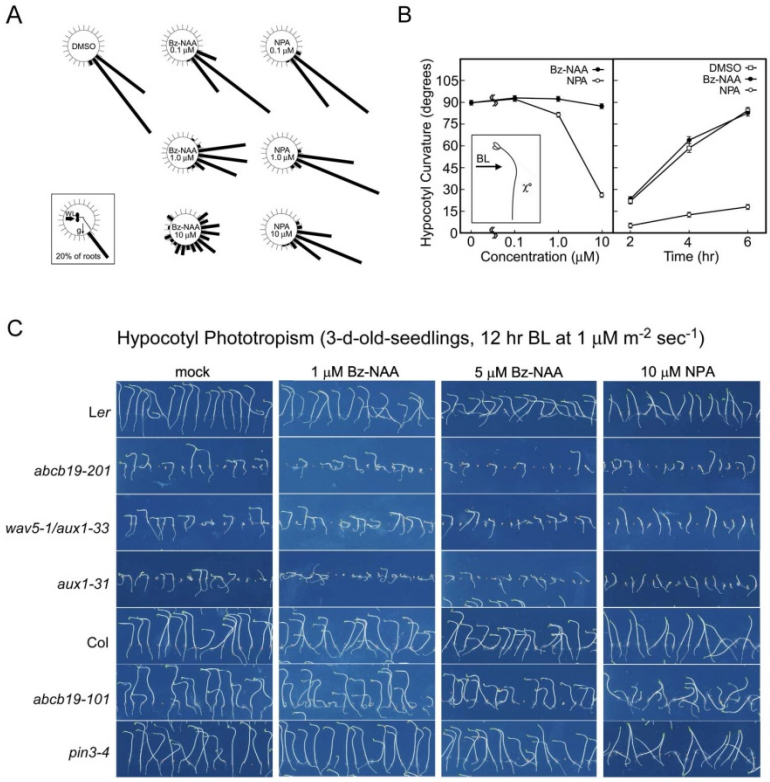


Fig. S4. Effects of Bz-NAA on phototropic response of *Arabidopsis* plant.
(A) Effect of Bz-NAA on root phototropism. Seedlings were irradiated by unilateral white light (thick arrow) at the fluence rate of 100 μmol m^{-2} sec^{-1} for 5 days. The frequency (%) of root growth direction at intervals of 15˚ are represented by the lengths of the bars. About 90 seedlings were measured in each experiment. Bz-NAA at 10 μM completely inhibited the phototropism of root. NPA at 10 μM reduced the phototropism of root, but the root still elongated toward the dark. (B) Effect of Bz-NAA on hypocotyl phototropism. Hypocotyl curvature of etiolated seedlings grown on vertical the half-strength Okada and Shimura (OS) agar medium with 7-Bz-NAA or NPA were measured after unilateral blue light irradiation at 1 mmol m^{-2} sec^{-1}. Indicated concentrations and 10 μM of inhibitors were used in left and right panels, respectively. 3d-old etiolated seedlings were irradiated with unilateral blue light for 6 hr and indicated time in left panel and right panels, respectively. (C) Photograph of the phototorpism of *Arabidopsis* wild-type: Landsberg *erecta* (Ler) and Columbia (Col) ecotypes, *aux1*, *pin3* and *abcb 19* mutants. Seeds' orientations were adjusted upwards as described previously (Nagashima et al., 2008). 3d-old seedlings grown on the half strength OS agar medium were irradiated with unilateral blue light for 12h at the fluence rate of 1 μmol m^{-2} sec^{-1}.

S5

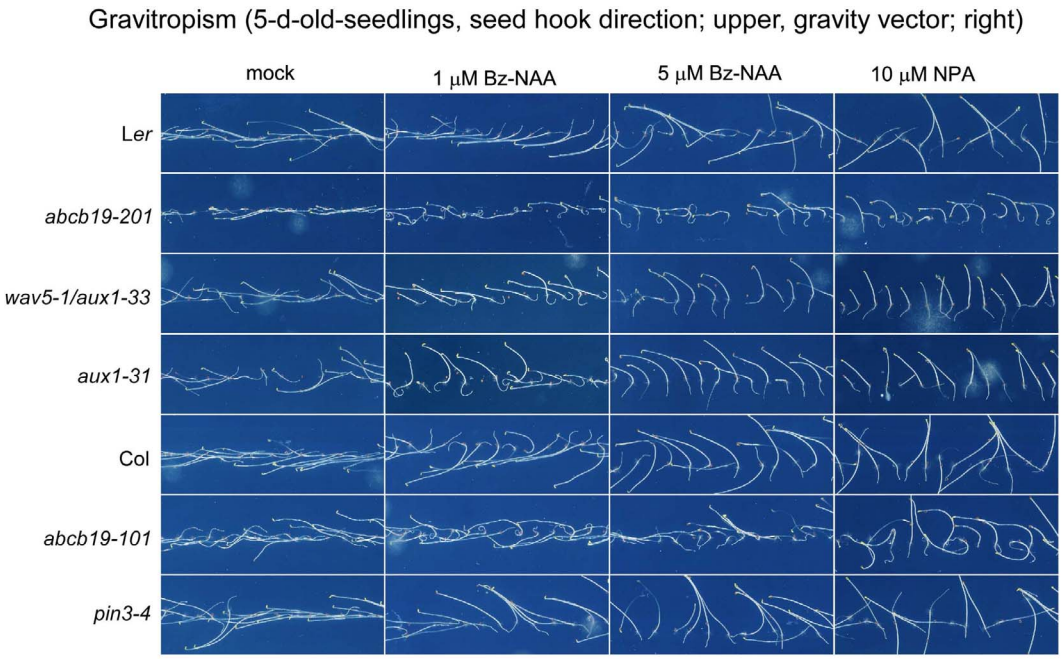


Fig. S5. Effects of Bz-NAA on gravitropic responses of *Arabidopsis* wild-type and auxin transport mutants. Photograph of the gravitropism of *Arabidopsis* wild-type, *aux1*, *pin3* and *abcb 19* mutants. Seeds' orientations were adjusted horizontally as described previously (Nagashima et al., 2008). *Arabidopsis* seedling wild-type: Landsberg *erecta* (Ler) and Columbia (Col) ecotypes, *aux1*, *pin3* and *abcb19* mutants were cultured for 5 days in dark on the OS agar medium containing Bz-NAA and NPA at indicated concentration.

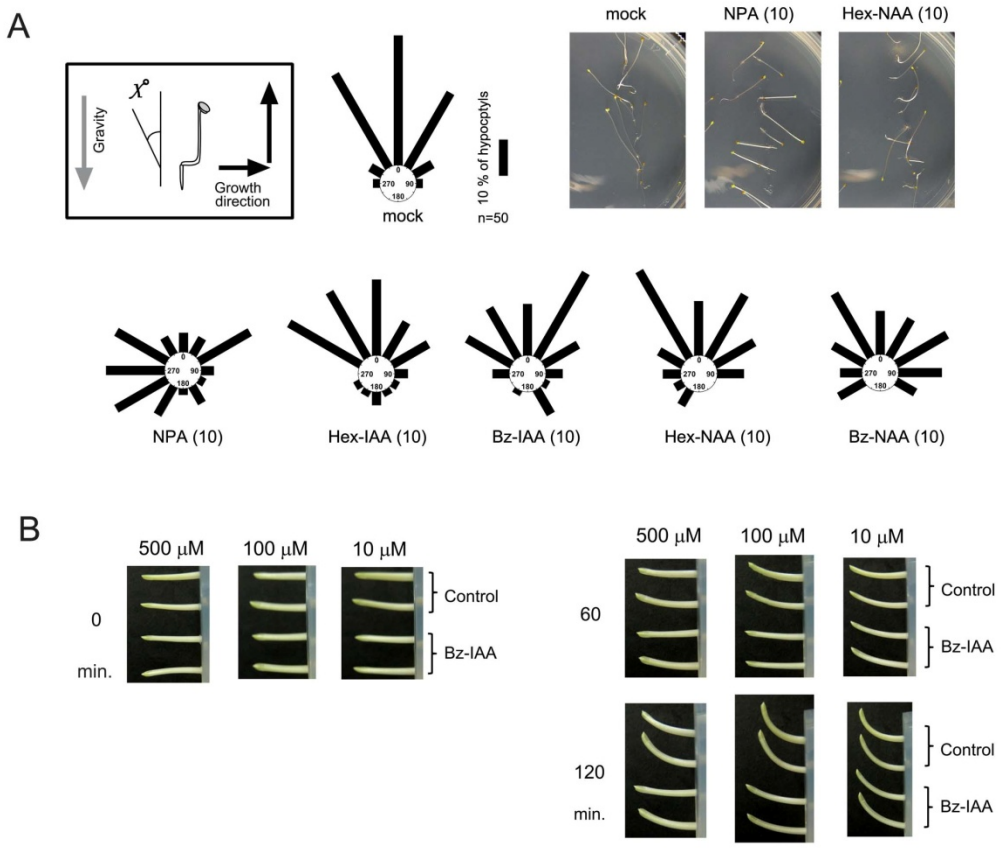


Fig. S6. Effects of alkoxy-auxins on the gravitropic responses of *Arabidopsis* hypocotyl and Maize coleoptile.

(A) Effects of alkoxy-auxin, Hex-IAA (**6a**), Bz-IAA (**7a**), Hex-NAA (**6b**), Bz-NAA (7**b**), on the hypocotyl gravitropism of *Arabidopsis* etiolated seedling. The orientation of seed was adjusted horizontally as described previously (Nagashima et al., 2008). The seedlings were grown on vertical GM agar medium with chemicals under dark condition. Angles (χ˚) of the hypocotyl-growth direction against gravity (g) of 5-d-old etiolated seedlings . The frequency (%) of hypocotyl growth direction at intervals of 30˚ are represented by the lengths of the bars. About 50 seedlings were measured in each experiment. The values in the parenthesis present the concentration of chemicals at μM.

(B) Effects of Bz-IAA (**7a**) on gravitropic curvature of Maize coleoptile segment (20 mm). The inhibitor solution at indicated concentration was loaded into the inside of 2 mm tip of coleoptile and then the coleoptile anchored on agar was cultured vertically for 30 min. The coleoptiles are tilted horizontally to observe the gravitropic response. Coleoptile photographs were taken at 60 min intervals.

S7

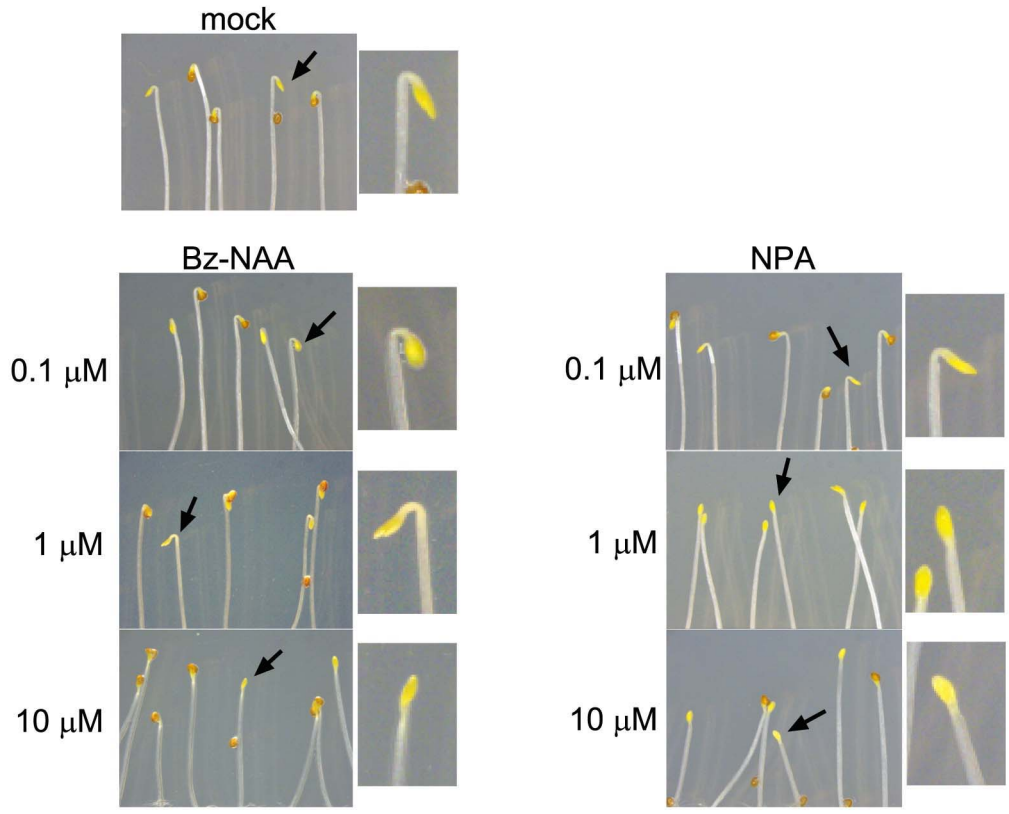


Fig S7. Effects of Bz-IAA on apical hook structure of *Arabidopsis* etiolated seedling.
Photograph of the hook structure of *Arabidopsis* etiolated seedling cultured vertically on OS agar medium containing inhibitors at indicated concentration in dark for 3 days. Bz-NAA (**7b**) at 10 μM induced the opening of apical hook. NPA at 1 μM induced the opening of apical hook. Arrows indicate the apical hook of seedlings.

Supplemental information:

Synthetic procedures of 5-alkoxy-indole 3-acetic acid and 7-alkoxy-naphthalene 1-acetic acid

Synthesis of chemicals

1.General experimental condition.

2. Synthetic scheme of 5-alkoxy-indole 3-acetic acid (**1a**–**7a**)

2.1. General synthetic procedure of 5-alkoxy IAA (**1a** – **7a**)

3. Synthetic scheme of 7-alkoxy-naphthalene 1-acetic acid (**1b**–**7b**)

3.1. General synthetic procedure of 7-alkoxy NAA (**1b** – **7b**)

SI References

Tang, J.D., Cen, J.D. (2009) Organic Preparations and Procedures International 41, 164-168

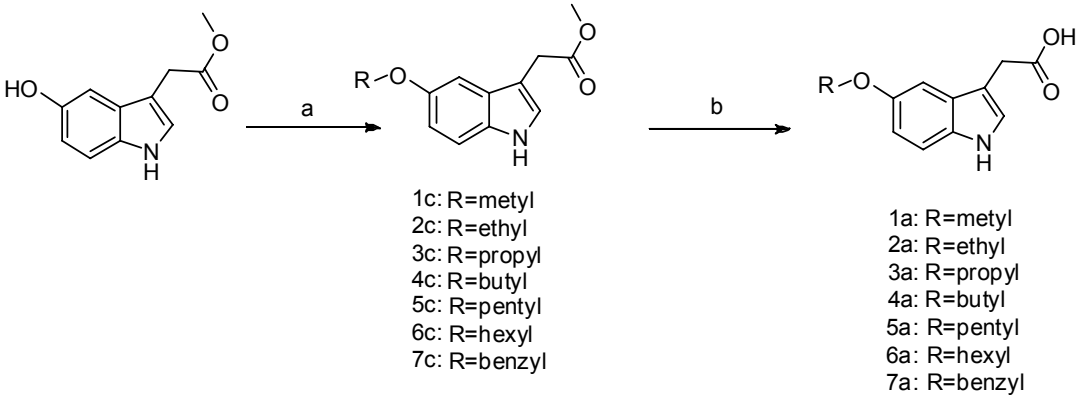
Silverman, R., Daub, G.H., VanderJagt, D. L. (1985) *J. Org. Chem.* 50, 5550-5556

Synthesis of chemicals

1. General experimental condition.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer (Bruker Japan, Japan). Chemical shifts are shown as δ values from TMS as the internal reference. Peak multiplicities are quoted in Hz. Mass spectra were measured on a JMS-700 spectrometer (JEOL, Japan). Column chromatography was carried out on columns of silica gel 60 (230–400 mesh, Merck, Japan). All chemicals were purchased from Tokyo Chemical Industry Japan (Tokyo, Japan), Wako Pure Chemical (Tokyo, Japan) and Sigma-Aldrich Japan (Tokyo, Japan) unless otherwise stated.

2. Synthetic scheme of 5-alkoxy-indole 3-acetic acid (1a-7a)



Scheme 1. *Reagents and conditions*: (a) R-I, Cs_2CO_3, DMF, 50˚C, 4h, (b) 2N aqueous NaOH: MeOH=1:2, rt, 1h.

2.1. General synthetic procedure of 5-alkoxy IAA (1a – 7b)

Starting material, 5-hydroxy-indole 3-acetic acid methyl ester was synthesized from 5-hydroxy-indole 3-acetic acid by Fisher esterfication (HCl in MeOH). To the solution of 5-hydroxy-indole 3-acetic acid methyl ester (100 mg, 0.5 mmol) in DMF (3 ml) was added cesium carbonate (162 mg, 0.5 equiv.) and corresponding alkyl iodide or aryl bromide (1.2 equiv.), and then stirred for 4h at 50˚C. The resulting solution was added to water (50 mL), and extracted with EtOAc (50 mL × 2). The organic layer was washed with saturated aqueousNH_4Cl solution and brine, and then dried over anhydrous Na_2SO_4. The residue was purified by a silica gel column chromatography to give the corresponding 5-alkoxy-indole-3-acetic acid methyl ester (**1c–7c**). This methyl ester was hydrolyzed in aqueous methanolic NaOH solution (2N NaOH : MeOH=1:2) at room temperature for 1h. The reaction solution was acidified to pH 3.5 with 6N HCl. After removal of MeOH *in vacuo*, the resulting suspension was extracted with EtOAc (50 mL × 3). The organic layer was washed successively with saturated aqueous

NH_4Cl solution and brine. After dried over anhydrous Na_2SO_4, the solvent was removed *in vacuo*. The residue was purified by a silica gel column chromatography to yield the corresponding 5-alkoxy-indole 3-acetic acid (**1a**-**7a**).

5-methoxy-indole 3-acetic acid (**1a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with methyl idodide, and then purified by a silica gel chromatography (hexane:acetone = 2:1) to yield 5-methoxy-indole 3-acetic acid methyl ester (**1c**: 72 % yield) as an brawn oil: IR υ max (neat): 3340, 2951, 1729, 1486, 1213, 1154 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.70 (3H, s), 3.74 (2H, s), 3.85(3H, s), 6.93(1H, dd, *J*=8.8, 2.3 Hz), 7.05 (1H, d, *J*=1.3 Hz), 7.11 (1H, d, *J*=2.3 Hz), 7.22 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 31.20, 51.94, 55.85, 100.56, 108.06, 111.92, 112.45, 123.81, 127.56, 131.20, 154.15, 172.52; EI-MS: *m/z* 219 $[M]^+$; HREI-MS: *m/z* 219.0886 $[M]^+$, calcd. for 219.0895 ($C_{12}H_{13}NO_3$).

The methyl ester (**1c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=6:1) to yield 5-methoxy-indole 3-acetic acid (**1a**: 81 % yield) as a brownish powder: m.p. 147–149 ˚C; IR υ max (neat): 3359, 2996, 2851, 1705, 1456, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.71 (2H, s), 3.80 (3H, s), 6.77 (1H, dd, *J*=8.8, 2.3 Hz), 7.11 (1H, d, *J*=2.3 Hz), 7.26 (1H, s), 7.28 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 31.45, 55.81, 101.35, 108.75, 112.39, 112.67, 125.16, 128.87, 132.59, 154.76, 173.33; EI-MS: *m/z* 205 $[M]^+$; HREI-MS: *m/z* 205.0737 $[M]^+$, calcd. for 205.0739 ($C_{11}H_{11}NO_3$).

5-ethoxy-indole 3-acetic acid (**2a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with ethyl iodide, and then purified by a silica gel chromatography (hexane : ethyl acetate = 7:3) to yield 5-ethoxy-indole 3-acetic acid methyl ester (**2c**: 81 % yield) as an oil; IR υ max (neat): 2978, 1729, 1474, 1211, 1154 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.45 (3H, t , *J*=7.0 Hz), 3.70 (3H, s), 3.75 (2H, s), 4.07 (2H, q, *J*=7.0 Hz), 6.87 (1H, dd, *J*=8.8, 2.3 Hz), 7.05 (1H, d, *J*=2.3 Hz), 7.12 (1H, d, *J*=1.9 Hz), 7.23 (1H, d, *J*=8.8 Hz) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 15.01, 31.22, 51.96, 64.17, 101.76, 108.12, 111.84, 113.02, 123.73, 127.62, 131.22, 153.41, 172.50; HREI-MS: *m/z* 233.1034 $[M]^+$, calcd. for 233.1052 ($C_{13}H_{15}NO_3$).

The methyl ester (**2c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-ethoxy-indole 3-acetic acid (**2a**: 97 % yield) as an brownish powder: m.p. 90–92 ˚C; IR υ max (neat): 3354, 3066, 2930, 1695, 1457, 1112 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (3H, t, *J*=7.0 Hz), 3.80 (2H, s), 4.09 (2H, q, *J*=7.0 Hz), 6.86 (1H, dd, *J*=8.8, 2.3 Hz), 7.04 (1H, d, *J*=2.3 Hz), 7.12 (1H, d, *J*=1.86 Hz), 7.23 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.99, 31.06, 64.18, 101.66, 107.66, 111.92, 113.17, 123.99, 127.51, 131.18, 153.48, 177.42; EI-MS: *m/z* (intensity %) 219

$[M]^+$, 205 (40 %), 190, 174, 162 (70 %), 160 (50 %); HREI-MS: *m/z* 219.0886 $[M]^+$, calcd. For 219.0895 ($C_{12}H_{13}NO_3$).

5-propoxy-indole 3-acetic acid (**3a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with 1-iodopropane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 3:1) to yield 5-propoxy-indole 3-acetic acid methyl ester (**3c**: 65 % yield) as a brownish oil: IR υ max (neat): 3355, 3061, 2961, 1695, 1457, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.07 (3H, t, *J*=6.7 Hz), 1.82 (2H, m), 3.70 (3H, s), 3.74 (2H, s), 4.01 (2H, t, *J*=6.7 Hz), 6.86 (1H, dd, *J*=8.8, 2.3 Hz), 7.05 (1H, d, *J*=2.3 Hz), 7.10 (1H, d, *J*=2.3 Hz), 7.21(1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 10.60, 22.75, 31.21, 51.95, 70.35, 101.74, 108.04, 111.83, 113.01, 123.74, 127.60, 131.19, 153.60, 172.54; EI-MS: *m/z* (intensity %) 247 $[M]^+$, 188 (30%), 149, 131 (75%); HREI-MS: *m/z* 247.1225 $[M]^+$, calcd. For 247.1208 ($C_{14}H_{17}NO_3$).

The methyl ester (**3c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-propoxy-indole 3-acetic acid (**3a**: 97 % yield) as an brownish powder: m.p. 134–137˚C; IR υ max (neat): 3407, 2954, 1728, 1456, 1213, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.05(3H, t, *J*=7.4 Hz), 1.82 (2H, m), 3.76 (3H, s), 3.96 (2H, t, *J*=6.6 Hz), 6.87 (1H, dd, *J*=8.1, 2.2 Hz), 7.04 (1H, d, *J*=2.2 Hz), 7.13 (1H, s), 7.23 (1H, d, *J*=8.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 10.60, 10.60, 22.75, 31.03, 70.35, 101.65, 107.46, 111.90, 113.20, 123.90, 127.53, 131.15, 153.72, 177.41; EI-MS: *m/z* (intensity %) 233 $[M]^+$, 191 (50%); HREI-MS: *m/z* 233.1043 $[M]^+$, calcd. for 233.1052 ($C_{13}H_{15}NO_3$).

5-butoxy-indole 3-acetic acid (**4a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with 1-iodobutane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 3:1) to yield 5-ethoxy-indole 3-acetic acid methyl ester (**4c**: 80 % yield) as an brownish oil; IR υ max (neat): 3355, 2956, 2922, 1694, 1459, 1127 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.98 (3H, t, *J*=7.4 Hz), 1.52 (2H, m), 1.82 (2H, m), 3.70 (3H, s), 3.74 (2H, s), 4.01 (2H, t, *J*=6.5 Hz), 6.86 (1H, dd, *J*=8.8, 2.3 Hz), 7.05 (1H, d, *J*=2.3 Hz), 7.09 (1H, d, *J*=2.1 Hz), 7.21 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.90, 19.31, 31.20, 31.94, 51.94, 68.50, 101.71, 108.02, 111.82, 113.00, 123.74, 127.59, 131.17, 153.62, 172.54; HREI-MS: *m/z* 261.1370 $[M]^+$, calcd. for 261.1365 ($C_{15}H_{19}NO_3$).

The methyl ester (**4c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-ethoxy-indole 3-acetic acid (**4a**: 88 % yield) as an brownish powder: m.p. 138–141 ˚C; IR υ max (neat): 3355, 2956, 2853, 1694, 1459, 1127 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.98 (3H, t, *J*=7.3 Hz), 1.51 (2H, m), 1.78 (2H, m), 3.76 (2H, s), 4.01 (2H, t, *J*=6.5 Hz), 6.87 (1H, dd, *J*=8.8, 2.0 Hz), 7.04 (1H, d, *J*=2.0 Hz), 7.14 (1H, s), 7.24 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.92, 19.32, 29.69, 31.55, 101.63, 107.51, 111.63, 113.22, 123.89, 131.15, 153.77, 173.29; EI-MS: *m/z* (intensity %) 247 $[M]^+$, 191(60%); HREI-MS: *m/z* 247.1189 $[M]^+$, calcd. for 247.1208

($C_{14}H_{17}NO_3$).

5-pentoxy-indole 3-acetic acid (**5a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with 1-iodopentane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 7:3) to yield 5-pentoxy-indole 3-acetic acid methyl ester (**5c**: 68 % yield) as a brownish oil; IR υ max (neat): 3396, 2951, 1732, 1455, 1213, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.94 (3H, t, *J*=7.2 Hz), 1.40 (2H, m), 1.46 (2H, m), 1.81 (2H, m), 3.07 (3H, s), 3.74 (2H, s), 4.00 (2H, t, *J*=6.7 Hz), 6.86 (1H, dd, *J*=8.8, 2.4 Hz), 7.05(1H, d, *J*=2.2 Hz), 7.09 (1H, d, *J*=2.4 Hz), 7.20 (1H, d, *J*=8.8 Hz) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.03, 22.50, 28.28, 29.23, 31.20, 51.94, 68.81, 101.70, 108.00, 111.83, 112.98, 123.75, 127.59, 131.18, 153.61, 172.55; EI-MS: *m/z* (intensity %) 261 $[M]^+$, 256 (60%), 247 (55%), 191 (85%); HREI-MS: *m/z* 275.1506 $[M]^+$, calcd. for 275.1521 ($C_{16}H_{21}NO_3$).

The methyl ester (**5c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-propoxy-indole 3-acetic acid (**5a**: 82 % yield) as an brownish powder: m.p. 128–132˚C; IR υ max (KBr): 3350, 3075, 2854, 1695, 1457, 1127 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, t, *J*=7.2 Hz), 1.8 (2H, m), 2.17 (2H, m), 3.75 (2H, s), 3.99 (2H, t, *J*=6.6 Hz), 6.86 (1H, dd, *J*=8.8, 2.3 Hz), 7.04 (1H, d, *J*=2.3 Hz), 7.12 (1H, s), 7.22 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.03, 19.32, 29.69, 31.55, 101.63, 107.51, 111.63, 113.22, 123.89, 131.15, 153.77, 173.29; EI-MS: *m/z* (intensity %) 261 $[M]^+$, 256 (80%), 228 (40%), 202, 191 (70%), 157 (85%), 191 (50%); HREI-MS: *m/z* 261.1304 $[M]^+$, calcd. for 261.1365 ($C_{15}H_{19}NO_3$).

5-hexyloxy-indole 3-acetic acid (**6a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with 1-iodohexane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 7:3) to yield 5-hexyloxy-indole 3-acetic acid methyl ester (**6c**: 78 % yield) as a brownish oil; IR υ max (neat): 3394, 2951, 1732, 1455, 1214, 1157 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, t, *J*=6.6 Hz), 1.35 (4H, m), 1.48 (2H, m), 1.80 (2H, m), 3.70 (3H, s), 3.74 (2H, s), 4.00 (2H, t, *J*=6.8 Hz), 6.86 (1H, dd, *J*=2.3, 8.7 Hz), 7.10 (1H, s), 7.05 (1H, *J*=2.3 Hz), 7.21 (1H, d, *J*=8.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.03, 22.61, 25.80, 29.45, 31.20, 31.64, 51.94, 68.83, 101.71, 108.71, 111.18, 113.01, 123.73, 127.61, 131.18, 153.63, 172.54; EI-MS: *m/z* (intensity %) 289 $[M]^+$, 246 (50%), 205 (35%), 177 (70%), 158 (100%), 146 (70%); HREI-MS: *m/z* 289.1662 $[M]^+$, calcd. for 289.1678 ($C_{17}H_{23}NO_3$).

The methyl ester (**6c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-hexyloxy-indole 3-acetic acid (**6a**: 79 % yield) as an yellow powder: m.p. 128–130˚C; IR υ max (KBr): 3350, 3075, 2924, 1695, 1455, 1127 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, t, *J*=6.9 Hz), 1.35 (4H, m), 1.50 (2H, m), 1.80 (2H, m), 3.75 (2H, s), 3.99 (2H, t, *J*=6.6 Hz), 6.86 (1H, dd, *J*=8.8, 2.2 Hz), 7.03 (1H, d, *J*=2.2 Hz), 7.11 (1H, s), 7.21 (1H, d, *J*=8.8 Hz); ^{13}C-NMR (100

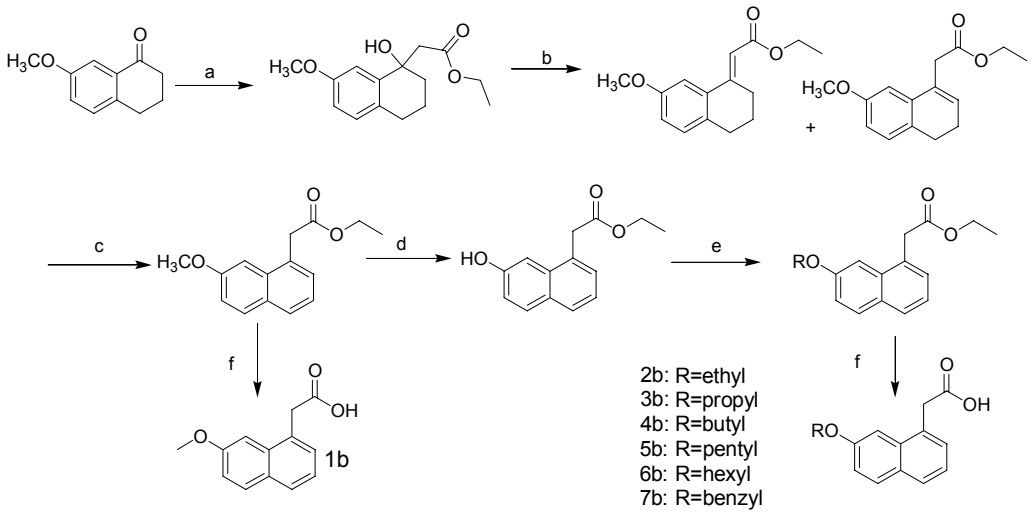
MHz, $CDCl_3$): δ 14.03, 22.61, 25.79, 29.18, 29.44, 31.04, 68.86, 101.65, 107.43, 111.89, 113.15, 123.92, 127.54, 131.16, 153.71, 177.55; EI-MS: *m/z* (intensity %) 275 $[M]^+$, 256 (60%), 230 (40%), 202 (75%), 191 (80%), 167 (40%), 157 (75%); HREI-MS: *m/z* 275.1477 $[M]^+$, calcd. for 275.1521 ($C_{16}H_{21}NO_3$).

5-benzyloxy-indole 3-acetic acid (**7a**)

Starting material 5-hydorxy-indole 3-acetic acid methyl ester (100 mg, 0.50 mmol) was reacted with benzyl bromide, and then purified by a silica gel chromatography (hexane:acetone = 3:1) to yield 5-benzyloxy-indole 3-acetic acid methyl ester (**7c**: 72 % yield) as a brownish oil; IR υ max (neat): 3395, 2951, 1732, 1486, 1213, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.66 (3H, s), 3.72 (2H, s), 5.10 (2H, s), 6.93 (1H, dd, *J*=8.8, 2.3 Hz), 7.07 (1H, d, *J*=2.2Hz), 7.13 (1H, d, *J*=2.3 Hz), 7.20 (1H, d, *J*=8.8 Hz), 7.27 (2H, d, *J*=7.2 Hz), 7.31 (1H, t, *J*=7.2 Hz), 7.38 (2H, t, *J*=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 31.20, 51.93, 70.85, 102.20, 108.04, 111.93, 113.09, 123.89, 127.52, 127.58 (2C), 127.73, 128.46 (2C), 131.38, 137.58, 153.31, 172.53; EI-MS: *m/z* (intensity %) 295 $[M]^+$, 284 (95%), 146 (55%); HREI-MS: *m/z* 295.1210 $[M]^+$, calcd. for 295.1208 ($C_{18}H_{17}NO_3$).

The methyl ester (**7c**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=9:1) to yield 5-propoxy-indole 3-acetic acid (**5a**: 82 % yield) as an brownish powder: m.p. 146–148˚C; IR υ max (KBr): 3359, 2919, 2851, 1728, 1458, 1150 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.75 (2H, s), 5.10 (2H, s), 6.94 (1H, dd, *J*=8.9, 2.3 Hz), 7.11 (1H, s), 7.13 (1H, d, *J*=2.3), 7.23 (1H, d, *J*=8.9), 7.29 (1H, t, *J*=7.3 Hz), 7.37 (2H, t, *J*=7.3 Hz), 7.46 (2H, d, *J*=7.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 31.02, 70.91, 102.22, 107.59, 111.97, 113.33, 124.02, 127.51, 127.64 (2C), 127.76, 128.47 (2C), 131.37, 137.55, 153.45, 177.42; EI-MS: *m/z* (intensity %) 281 $[M]^+$, 256 (65%), 191 (20%), 167 (32%); HREI-MS: *m/z* 281.1042 $[M]^+$, calcd. for 281.1052 ($C_{15}H_{19}NO_3$).

3. Synthetic scheme of 7-alkoxy-naphthalene 1-acetic acid (1b-7b)



Scheme 1. *Reagents and conditions*: (a) LHMDS, Ethyl acetate in THF, - 70 ˚C, 4h, (b) TFA in toluene, 80˚C, 1h, (c) DDQ in toluene, 80˚C, 2h, (d) BBr_3 in CH_2Cl_2, – 70 ˚C, 2h (e) Cs_2CO_3, DMF, rt, 4h, (f) 2N aqueous NaOH: MeOH=1:2, rt, 2h.

3.1. General synthetic procedure of 7-alkoxy NAA (1b – 7b)

Common starting material, 7-hydroxy-naphthalene 1-acetic acid ethyl ester was synthesized from 7-methoxy-tetralone according to reported procedures shown in scheme 2 (J.D. Tang et.al. 2009; R. Silverman et al., 1985). To the solution of 7-hydroxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) in DMF (2 ml) was added cesium carbonate (70 mg, 1 equiv.) and corresponding alkyl iodide or aryl bromide (2 equiv.), and then stirred for room temperature for 4h. The resulting solution was added to water (20 mL), and extracted with EtOAc (20 mL × 2). The organic layer was washed with saturated aqueous NH_4Cl solution and brine, and then dried over anhydrous Na_2SO_4. The residue was purified by a silica gel column chromatography to give the corresponding 7-alkoxy-naphthalene 1-acetic acid ethyl ester (**2d–7d**). This ethyl ester was hydrolyzed in aqueous methanolic NaOH solution (2N NaOH : MeOH=1:2) at room temperature for 2h. The reaction solution was acidified to pH 3.5 with 6N HCl. After removal of MeOH *in vacuo*, the resulting suspension was extracted with EtOAc (20 mL × 3). The organic layer was washed successively with saturated aqueous NH_4Cl solution and brine. After dried over anhydrous Na_2SO_4, the solvent was removed *in vacuo*. The residue was purified by a silica gel column chromatography to yield the corresponding 7-alkoxy-naphthalene 1-acetic acid (**1b–7b**). For the synthesis of 7-methoxy-naphthalene 1-acetic acid, the intermediate, 7-methoxy-naphthalene 1-acetic acid ethyl ester was hydrolyzed in aqueous methanolic NaOH solution (2N NaOH : MeOH=1:2) at room temperature for

2h to give a 7-methoxy-naphthalene 1-acetic acid.

7-methoxy-naphthalene 1-acetic acid (**1b**)

Starting material 7-methoxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was hydrolyzed in aqueous methanolic NaOH solution and then extracted with EtOAc at pH 3. The organic layer was concentrated in vacuo and purified by a silica gel chromatography ($CHCl_3$:MeOH = 10:1) to yield 7-methoxy-naphthalene 1-acetic acid (**1b**: 73 % yield) as an colorless powder: m.p. 145–149˚C; IR υ max (neat): 3400-2700 (broad OH), 2925, 1694, 1626, 1265, 1232cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (3H, s), 4.00 (2H, s), 7.15 (1H, dd, J=2.2, 8.9 Hz), 7.22 (1H, d, *J*=2.2 Hz), 7.29 (1H, dd, *J*=7.4, 8.4 Hz), 7.36 (1H, d, *J*=7.4 Hz), 7.72 (1H, d, *J*=8.4 Hz), 7.75 (1H, d, *J*=8.9 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.26, 55.25, 102.32, 118.39, 123.18, 128.01, 128.55, 128.75, 129.22, 130.28, 133.15, 158.10, 177.55; EI-MS: *m/z* 216 $[M]^+$, 171 (100); HREI-MS: *m/z* 216.0755 $[M]^+$, calcd. for 216.0786 ($C_{13}H_{12}O_3$).

7-ethoxy-naphthalene 1-acetic acid (**2b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with ethyl iodide, and then purified by a silica gel chromatography (hexane:acetone = 5:1) to yield 7-ethoxy–naphthalene 1-acetic acid ethyl ester (**2d**: 95 % yield) as an colorless powder; IR υ max (neat): 2926, 1729, 1260, 1204, 1144 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (3H, t, *J*=7.0), 1.49 (3H, t, *J*=7.0), 4.00 (2H, s), 4.15 (4H, m), 7.15 (1H, dd, *J*=2.0, 8.9), 7.28 (2H, m), 7.37 (1H, d, *J*=6.8 Hz), 7.71 (1H, d, *J*=8.0 Hz), 7.75 (1H, d, *J*=8.9 Hz); EI-MS: *m/z* (intensity %) 258 $[M]^+$, 185 (70 %), 157 (60%), 128 (20 %); HREI-MS: *m/z* 258.1255 $[M]^+$, calcd. for 258.1256 ($C_{16}H_{18}NO_3$).

The ethyl ester (**2d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH = 10:1) to yield 5-ethoxy-naphthalene 1-acetic acid (**2b**: 98 % yield) as an colorless powder: m.p. 156 – 157 ˚C; IR υ max (neat): 3400-2700 (broad OH), 2944, 1694, 1626, 1259, 1206cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (3H, t, *J*=7.0 Hz), 3.97 (2H, s), 4.09 (2H, q, *J*=7.0 Hz), 7.14 (1H, dd, *J*=9.0, 2.0 Hz), 7.20 (1H, d, *J*=2.0 Hz), 7.25 (1H, t, *J*=8.0, 6.8 Hz), 7.33 (1H, d, *J*=6.8 Hz), 7.70 (1H, d, *J*=8.0 Hz), 7.74 (1H, d, *J*=9.0 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.66, 39.28, 63.44, 103.06, 118.64, 123.64, 127.96, 128.54, 128.66, 129.11, 130.20, 133.16, 157.38, 177.65; EI-MS: *m/z* (intensity %) 230 $[M]^+$, 202, 185, 173, 157; HREI-MS: *m/z* 230.0922 $[M]^+$, calcd. for 230.0943 ($C_{14}H_{14}NO_3$).

7-propoxy-naphthalene 1-acetic acid (**3b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with 1-iodopropane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 20:1) to yield 7-propoxy-naphthalene 1-acetic acid ethyl ester (**3d**: 91 % yield) as an colorless powder; m.p. 39–42 ˚C; IR υ max (neat): 2970, 1734, 1627 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07 (3H, t, *J*=7.0),

1.21(3H, t, *J*=7.1), 1.87 (2H, m), 3.97 (2H, s), 4.03 (2H, t, *J*=7.0), 4.13 (2H, q, *J*=7.1), 7.15 (1H, dd, *J*=9.0, 2.0), 7.26 (2H, m), 7.35 (1H, d, *J*=6.8), 7.68 (1H, d, *J*=8.0), 7.72 (1H, d, *J*=9.0); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 10.54, 14.13, 22.48, 39.64, 60.81, 69.42, 103.24, 118.55, 122.99, 127.60, 128.37, 129.09, 129.30, 130.04, 133.25, 157.45, 171.56; EI-MS: *m/z* (intensity %) 272 $[M]^+$, 230 (70 %), 199 (25%), 157 (100 %); HREI-MS: *m/z* 272.1411 $[M]^+$, calcd. for 272.1412 ($C_{17}H_{20}O_3$).

The ethyl ester (**3d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=10:1) to yield 7-propoxy-naphthalene 1-acetic acid (**3b**: 87 % yield) as an colorless powder: m.p. 125–127 ˚C; IR υ max (neat): 3400-2700 (broad OH), 2960, 1692, 1617, 1259, 1208 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, t, *J*=7.4), 1.84 (2H, m), 4.00 (2H, s), 4.00 (2H, t, *J*=6.5), 7.16 (1H, dd, *J*=9.0, 2.2), 7.22 (1H, d, *J*=2.2), 7.27 (1H, dd, *J*=8.1, 6.5), 7.36 (1H, d, *J*=6.5), 7.71 (1H, d, *J*=8.1), 7.74 (1H, d, *J*=9.0);^{13}C-NMR (100 MHz, $CDCl_3$): δ 10.53, 22.46, 39.15, 69.49, 103.08, 118.73, 123.02, 128.00, 128.40, 128.65, 129.13, 130.19, 133.18, 157.62, 177.66; EI-MS: *m/z* (intensity %) 244 $[M]^+$, 202, 157; HREI-MS: *m/z* 244.1119 $[M]^+$, calcd. for 244.1099 ($C_{15}H_{16}O_3$).

7-butoxy-naphthalene 1-acetic acid (**4b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with 1-iodobutane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 4:1) to yield 7-butoxy-naphthalene 1-acetic acid ethyl ester (**4d**: 84 % yield) as an colorless oil; IR υ max (neat): 2958, 1733, 1625 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, *J*=7.5), 1.19 (3H, t, *J*=7.1), 1.53 (2H, m), 1.82 (2H, m), 3.97 (2H, s), 4.07 (2H, t, *J*=6.6), 4.12 (2H, q, *J*=7.1), 7.14 (1H, dd, *J*=8.9, 2.3), 7.25 (1H, dd, *J*=8.1, 6.9), 7.27 (1H, d, *J*=2.3), 7.35 (1H, d, *J*=6.9), 7.67 (1H, d, *J*=8.1), 7.71 (1H, d, *J*=8.9); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.79, 14.10, 19.24, 31.20, 39.52, 60.77, 67.57, 103.19, 118.54, 122.96, 127.58, 128.34, 129.08, 129.28, 130.01, 133.22, 157.44, 171.52; EI-MS: *m/z* (intensity %) 286 $[M]^+$, 230 (65 %), 157 (100 %); HREI-MS: *m/z* 286.1556 $[M]^+$, calcd. for 286.1569 ($C_{18}H_{22}O_3$).

The ethyl ester (**4d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=10:1) to yield 7-butoxy-naphthalene 1-acetic acid (**4b**: 97 % yield) as colorless powder: m.p. 102–104 ˚C; IR υ max (neat): 3400-2800 (broad OH), 2930, 1699, 1620, 1260, 1206 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (3H, t, *J*=7.4), 1.51 (2H, m), 1.80 (2H, m), 4.00 (2H, s), 4.05 (2H, t, *J*=6.5), 7.16 (1H, dd, *J*=8.9, 2.0), 7.23 (1H, d, *J*=2.0), 7.26 (1H, dd, *J*=8.1, 6.9), 7.34 (1H, d, *J*=6.9), 7.71 (1H, d, *J*=8.1), 7.75 (1H, d, *J*=8.9);^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.84, 19.27, 31.20, 39.20, 67.70, 103.14 , 118.68, 123.01, 127.92 (2 carbon signals), 128.59, 129.11, 130.16, 133.19, 157.61, 177.58; EI-MS: *m/z* (intensity %) 258 $[M]^+$, 202, 157; HREI-MS: *m/z* 258.1268 $[M]^+$, calcd. for 258.1256 ($C_{16}H_{18}O_3$).

7-pentoxy-naphthalene 1-acetic acid (**5b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with 1-iodopentane, and then purified by a silica gel chromatography (hexane:ethyl acetate = 7:3)

to yield 7-pentoxy-naphthalene 1-acetic acid ethyl ester (5**d**: 93 % yield) as an colorless oil; IR υ max (neat): 2969, 1734, 1624 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.00 (3H, t, *J*=7.2), 1.26 (3H, t, *J*=7.1), 1.48 (2H, m), 1.55 (2H, m), 1.91 (2H, m), 4.03 (2H, s), 4.13 (2H, t, *J*=6.5), 4.19 (2H, q, *J*=7.1), 7.20 (1H, dd, *J*=8.9, 2.5), 7.31 (1H, dd, *J*=8.1, 7.0), 7.33 (1H, d, *J*=2.5), 7.41 (1H, d, *J*=7.0), 7.74 (1H, d, *J*=8.1), 7.78 (1H, d, *J*=8.9);^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.95, 14.11, 22.42, 28.22, 28.85, 39.62, 60.78, 67.78, 103.20, 118.54, 122.96, 127.58, 128.35, 129.08, 129.28, 130.02, 133.23, 157.44, 171.53; EI-MS: *m/z* (intensity %) 300 $[M]^+$, 230 (65 %), 157 (100 %); HREI-MS: *m/z* 300.1727 $[M]^+$, calcd. for 300.1725 ($C_{19}H_{24}O_3$).

The ethyl ester (**5d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=10:1) to yield 7-butoxy-naphthalene 1-acetic acid (**5b**: 93 % yield) as colorless powder: m.p. 104 – 106 ˚C; IR υ max (neat): 3400-2800 (broad OH), 2945, 1689, 1620, 1257, 1209 cm^{-1}; ^1H-NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, t, *J*=7.1), 1.39 (2H, m), 1.45 (2H, m), 1.82 (2H, m), 3.87(1H, d, *J*=8.9), 4.00 (2H, s), 4.03 (2H, t, *J*=6.5), 7.15（1H, dd, *J*=8.9,2.1）, 7.21 (1H, d, *J*=2.1), 7.26 (1H, t, *J*=8.1, 6.9), 7.39 (1H, d, *J*=6.9), 7.71（1H, d, *J*=8.1）;^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.00, 22.46, 28.23, 28.85, 39.13, 68.01, 103.07, 118.73, 123.01, 128.00, 128.40, 128.64, 129.12, 130.19, 133.18, 157.64, 177.64; EI-MS: *m/z* (intensity %) 272 $[M]^+$, 202, 157; HREI-MS: *m/z* 272.1378 $[M]^+$, calcd. for 272.1412 ($C_{17}H_{20}O_3$).

7-hexyloxy-naphthalene 1-acetic acid (**6b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with 1-iodohexane, and then purified by a silica gel chromatography (hexane : ethyl acetate = 6:1) to yield 7-hexyloxy-naphthalene 1-acetic acid ethyl ester (6**d**: 92 % yield) as an colorless oil; IR υ max (neat): 2932, 1733, 1509, 1459, 1211, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.93 (3H, t, *J*=7.0), 1.22 (3H,t,J=7.1), 1.38 (4H, m), 1.51 (2H, m), 1.85 (2H, m), 3.99 (2H, s), 4.08 (2H, t, *J*=6.5), 4.15 (2H, q. *J*=7.1), 7.12(1H, dd, *J*=8.9, 2.5), 7.26 (1H, d, *J*=2.5), 7.26 (1H, dd, *J*=8.1,6.9), 7.37 (1H, d, *J*=6.9), 7.69 (1H, d, *J*=8.1), 7.75 (1H, d, *J*=8.9);^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.03, 14.20, 22.60, 25.80, 29.19, 31.61, 39.71, 60.89, 67.99, 103.23, 118.62, 123.04, 127.66, 128.42, 129.12, 129.34, 130.09, 133.27, 157.50, 171.64; EI-MS: *m/z* (intensity %) 314 $[M]^+$, 230 (65 %), 150, 131 (30%); HREI-MS: *m/z* 314.1870 $[M]^+$, calcd. for 314.1882 ($C_{20}H_{26}O_3$).

The ethyl ester (**6d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=10:1) to yield 7-butoxy-naphthalene 1-acetic acid (**5b**: 97 % yield) as colorless powder: m.p. 103–105 ˚C; IR υ max (neat): 3400-2800 (broad OH), 2921, 1700, 1257, 1209 cm^{-1}; ^1H-NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, t, J=7.0), 1,47 (2H, m), 1.34(4H, m), 1.81 (2H, m), 4.02 (2H, s), 4.03 (2H, t, *J*=6.5), 7.15 (1H, dd, *J*=8.9, 2.2), 7.21 (1H, d, *J*=2.2), 7.27 (1H, d, *J*=8.1, 6.6), 7.36 (1H. d, *J*=6.6), 7.71 (1H, d, *J*=8.1), 7.74 (1H, d, *J*=8.9);^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.04, 22.60, 25.77, 29.13, 31.61, 39.15, 68.02, 103.04, 118.73, 123.01, 128.01, 128.38, 128.65, 129.11, 130.19, 133.18, 157.64, 177.79; EI-MS: *m/z* (intensity %) 286 $[M]^+$, 202, 157; HREI-MS: *m*/z 286.1568 $[M]^+$, calcd. for 286.1569

($C_{18}H_{22}O_3$).

7-hexyloxy-naphthalene 1-acetic acid (**7b**)

Starting material 7-hydorxy-naphthalene 1-acetic acid ethyl ester (50 mg, 0.21 mmol) was reacted with benzylbromide and then purified by a silica gel chromatography (hexane:ethyl acetate = 6:1) to yield 7-benzyloxy-naphthalene 1-acetic acid ethyl ester (7**d**: 92 % yield) as an colorless oil; IR υ max (neat): 2931, 1734, 1509, 1456, 1211, 1159 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (3H, t, *J*=7.2), 3.96 (2H, s), 4.11 (2H, q, *J*=7.2), 5.17 (2H, s), 7.19 – 7.40 (8H, m), 7.48 (1H, d, *J*=7.2), 7.69 (1H, d, *J*=8.0), 7.74 (1H, d, *J*=8.9);^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.18, 39.63, 60.86, 69.98, 103.90, 118.59, 123.26, 127.62 (3C), 127.69, 128.47, 128.54 (2C), 129.29, 129.42, 130.20, 133.16, 136.76, 157.06, 171.50; EI-MS: *m/z* (intensity %) 320 $[M]^+$, 279 (25%), 230 (20%); HREI-MS: *m/z* 320.1434 $[M]^+$, calcd. for 314.1412 ($C_{21}H_{20}O_3$).

The ethyl ester (**7d**) was alkaline hydrolyzed and then purified by a silica gel chromatography ($CHCl_3$:MeOH=20:1) to yield 7-hexyloxy-naphthalene 1-acetic acid (**7b**: 98 % yield) as colorless powder: m.p. 133–136 ˚C; IR υ max (neat): 3400-2800 (broad OH), 2921, 1695, 1257, 1191 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.95 (2H, s), 5.13 (2H, s), 7.22 (1H, dd, *J*=8.9, 2.2), 7.23 – 7.34 (6H, m), 7.43 (2H, d, *J*=7.2), 7.70 (1H, d, *J*=7.5), 7.74 (1H, d, *J*=8.9); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 39.12 , 70.02 , 103.82 , 118.77 , 123.25 , 127.58 (2C), 127.96 , 128.00 , 128.48 , 128.53 (2C), 128.74 , 129.27 , 130.31 , 133.05 , 136.67 , 157.15 , 177.84; EI-MS: *m/z* (intensity %) 292 $[M]^+$, 248(15%), 202(20%), 157(20%), 128(30%); HREI-MS: *m*/z 292.1132 $[M]^+$, calcd. for 292.1099 ($C_{19}H_{16}O_3$).