Supplementary Information

Design, Synthesis and Biological Evaluation of 4'-Demethyl-4-Deoxypodophyllotoxin Derivatives as Novel Tubulin and Histone Deacetylase Dual Inhibitors

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General experimental

Melting points were taken on a Fisher–Johns melting point apparatus, uncorrected and reported in degrees Centigrade. ^{1}H NMR spectra and ^{13}C NMR were recorded in $CDCl_3$, D_2O and DMSO–d_6 on a Bruker DRX–400 (400 MHz) using TMS as internal standard. Chemical shifts were reported as δ (ppm) and spin–spin coupling constants as *J* (Hz) values. The mass spectra (MS) were recorded on a Finnigan MAT–95 mass spectrometer. The purity of all tested compounds was established by HPLC to be >95.0%. HPLC analysis was performed at room temperature using an Agilent Eclipse XDB–C18 (250 mm × 4.6 mm) and as a mobile phase gradient from 10% MeOH/H_2O (1‰ AcOH) for 1 min, 10% MeOH/H_2O (1‰ AcOH) to 60% MeOH/H_2O (1‰ AcOH) for 6 min, 60% MeOH/H_2O (1‰ AcOH) to 90% MeOH/H_2O (1‰ AcOH) for 6 min and 90% MeOH/H_2O (1‰ AcOH) for 5 min more, a flow rate of 1.0 mL/min. and plotted at 254 nm.

Functional assay

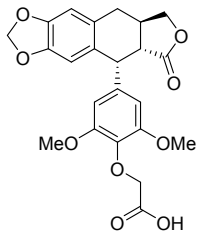
HDAC enzymatic assay in vitro

Recombinant human HDAC1, HDAC2 and HDAC3 were cloned and expressed in High5 insect cells using a baculovirus expression system and purified using Ni–NTA (QIAGEN). The histone deacetylase inhibitory activity was determined using the HDAC substrate Ac–Lys–Tyr–Lys (ε–acetyl)–AMC. The reaction was carried out in black 384–well plates (OptiPlateTM–384F, PerkinElmer) at room temperature. The typical inhibition assay was carried out in 25 μL of buffer containing 25 mM HEPES, 137 mM NaCl, 2.7 mM KCl and 4.9 mM $MgCl_2$, pH 8.0, HDAC protein (20–200 nM), HDAC substrate (5–50 μM) and 20 μg/mL individual compound. Positive controls contained MGCD0103 and all the above components except the inhibitor. The negative controls contained neither enzyme nor inhibitor. After incubation for 24 h and 3 h respectively, the reaction of HDAC1, HDAC2 and HDAC3 was quenched with the addition of 25 μL Trypsin (diluted to a final concentration of 0.3125%). The plates were incubated for 30 min at room temperature to allow the fluorescence signal to develop. The fluorescence generated was monitored at 355nm (excitation) and 460nm (emission) using an EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA, USA).

Cell culture and Cytotoxicity/proliferation assay

Cells were kept at logarithmic growth phase in 5% CO_2 at 37 °C with the corresponding medium supplemented with 10% fetal bovine serum and 100 units/mL each of penicillin G and streptomycin. Cells were seeded onto a 96–well plate at a concentration of 2000–3000 cells/well and incubated at 37 °C in 5% CO_2 for 24 h. A range of concentrations of the test compounds were added and the plate was incubated at 37 °C for 72 h before 20 μL MTT (5 mg/mL)/well was added. After 3 h of incubation, the medium was removed and 100 μL DMSO was added to each well. The absorbance was measured using a SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 550 nm with a reference at 690 nm. The optical density of the result of the MTT assay was directly proportional to the number of viable cells.

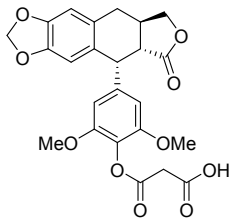
Preparation and analytical data



Chemical Formula: $C_{23}H_{22}O_9$
Exact Mass: 442.1264

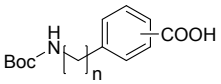
Compound 3

DDPT (384 mg, 1.0 mmol), tert-butyl 2-bromoacetate (600 mg, 3.0 mmol), K_2CO_3 (150 mg, 1.1 mmol) and KI (20 mg, 0.1 mmol) were stirred in 20 mL DMF at 80 °C for 24 h. The reaction mixture was cooled to room temperature and then extracted with DCM. The organic layer was washed with water followed by brine. The solution was dried over Na_2SO_4 and then treated with 0.5 mL trifluoroacetic acid. The mixture was stirred at room temperature for 5 h and extracted with DCM. The organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified by column chromatography to give pure compound **3** as a white solid (206 mg, 47%). mp 94–96 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (s, 1H), 6.49 (s, 1H), 6.40 (s, 2H), 5.96 (d, J = 10.0 Hz, 2H), 4.62 (d, J = 2.8 Hz, 1H), 4.57 (s, 2H), 4.47 (t, J = 7.6 Hz, 1H), 3.94 (t, J = 9.5 Hz, 1H), 3.79 (s, 6H), 3.16 – 3.04 (m, 1H), 2.85 – 2.60 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.83, 170.67, 151.09, 147.23, 146.89, 137.99, 135.24, 130.04, 128.36, 110.33, 108.59, 108.03, 101.29, 72.05, 71.17, 56.27, 47.37, 43.75, 33.05, 32.84; MS (ESI–) m/z = 441.2 ($M - H^+$).



Compound 4

2,2-Dimethyl-1,3-dioxane-4,6-dione (144 mg, 1.0 mmol) and DDPT (384 mg, 1.0 mmol) were refluxed in 15 mL toluene for 5 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was further purified by column chromatography (DCM:MeOH = 50:1) to give pure compound **4** as a colorless slurry (160 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 1H), 6.51 (s, 1H), 6.37 (s, 2H), 5.96 (dd, J = 8.0, 1.2 Hz, 2H), 4.61 (d, J = 3.6 Hz, 1H), 4.44 (dd, J = 8.5, 6.3 Hz, 1H), 3.94-3.87 (m, 2H), 3.80-3.76 (m, 1H), 3.68 (s, 6H), 3.06 (dd, J = 14.2, 3.5 Hz, 1H), 2.80-2.67 (m, 3H); MS (ESI+) m/z = 493.0 ($M + Na^+$).



6a-d

a *para*-substitute, n=1
b *meta*-substitute, n=1
c *para*-substitute, n=0
d *meta*-substitute, n=0

Representative procedure for 6a-d

Amino acids **5a-d** (1.0 equiv) was dissolved in a mixture of THF and water. Then it was treated with Boc_2O (2.0 equiv) in THF and $NaHCO_3$ (3.0 equiv) in water. The mixture was stirred at room temperature for 12 h followed by acidization with 1N HCl (aq) till pH = 3. The solution was extracted with ethyl acetate and the organic layer was concentrated under reduced pressure. The crude product was crystallized in a mixture of ethyl acetate and petroleum ether to give the target molecules.

4-(((tert-Butoxycarbonyl)amino)methyl)benzoic acid (6a)

Starting from **5a**, compound **6a** (3.08 g, 93%) was obtained as a white solid according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 4.94 (s, 1H), 4.40 (d, *J* = 5.2 Hz, 2H), 1.47 (s, 9H).

3-(((tert-Butoxycarbonyl)amino)methyl)benzoic acid (6b)

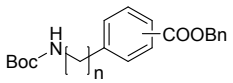
Starting from **5b**, compound **6b** (840 mg, 63%) was obtained as a white solid according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.95 (m, 2H), 7.58 – 7.50 (m, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 4.93 (s, 1H), 4.39 (d, *J* = 5.1 Hz, 2H), 1.47 (s, 9H).

4-((tert-Butoxycarbonyl)amino)benzoic acid (6c)

Starting from **5c**, compound **6c** (2.24 g, 65%) was obtained as a white solid according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.7 Hz, 2H), 6.74 (s, 1H), 1.54 (s, 9H).

3-((tert-Butoxycarbonyl)amino)benzoic acid (6d)

Starting from **5d**, compound **6d** (2.0 g, 84%) was obtained as a white solid according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 6.67 (s, 1H), 1.54 (s, 9H).

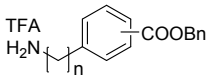


7a-d

a *para*-substitute, n=1
b *meta*-substitute, n=1
c *para*-substitute, n=0
d *meta*-substitute, n=0

Representative procedure for 7a-d

A mixture of compounds **6a-d** (1.0 equiv), BnBr (1.0 equiv) and $CsCO_3$ (1.05 equiv) in DMF was stirred at room temperature for 3 h. Then it was extracted with ethyl acetate and the organic layer was washed twice with water, dried over Na_2SO_4 and concentrated in vacuo. The crude product was used directly in the next step.



8a-d

a *para*-substitute, n=1
b *meta*-substitute, n=1
c *para*-substitute, n=0
d *meta*-substitute, n=0

Representative procedure for 8a-d

Compounds **7a-d** (1.0 equiv) were dissolved in DCM and treated with trifluoroacetic acid (5.0 equiv). The mixture was stirred at room temperature for 12 h and then concentrated in vacuo. The crude product was washed with diethyl ether to give corresponding aromatic amine.

Benzyl 4-(aminomethyl)benzoate (8a)

Starting from 1.0 g **6a**, compound **8a** (1.16 g, 86%, 2 steps) was obtained as a white solid according to above–mentioned general procedure. mp 126–128 °C, ^{1}H NMR (400 MHz, D_2O) δ 8.02 (d, *J* = 8.3 Hz, 2H), 7.54 – 7.35 (m, 7H), 5.34 (s, 2H), 4.20 (s, 2H); MS (ESI+) m/z = 242.2 ($M + H^+$).

Benzyl 3-(aminomethyl)benzoate (8b)

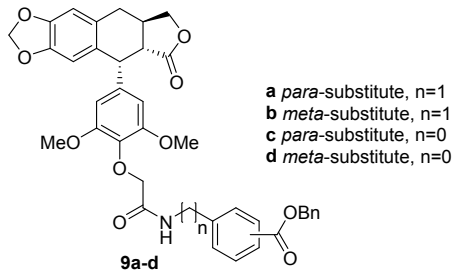
Starting from 890 mg **6b**, compound **8b** (1.03 g, 82%, 2 steps) was obtained as a white solid according to above–mentioned general procedure. mp 141–143 °C, ^{1}H NMR (400 MHz, DMSO) δ 8.26 – 8.10 (m, 4H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.52 – 7.34 (m, 5H), 5.39 (s, 2H), 4.13 (s, 2H); MS (ESI+) m/z = 242.2 ($M + H^+$).

Benzyl 4-aminobenzoate (8c)

Starting from 2.24 g **6c**, compound **8c** (2.95 g, 92%, 2 steps) was obtained as a white solid according to above–mentioned general procedure. mp 126–128 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 2H), 7.47 – 7.30 (m, 5H), 6.70 (d, *J* = 8.6 Hz, 2H), 5.33 (s, 2H); MS (ESI+) m/z = 228.2 ($M + H^+$).

Benzyl 3-aminobenzoate (8d)

Starting from 1.43 g **6d**, compound **8d** (1.60 g, 78%, 2 steps) was obtained as a white solid according to above–mentioned general procedure. mp 119–122 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.30 (m, 8H), 6.93 (d, *J* = 7.5 Hz, 1H), 5.35 (s, 2H); MS (ESI+) m/z = 228.1 ($M + H^+$).



Representative procedure for 9a-d

A mixture of compound **3** (1.0 equiv), HATU (1.05 equiv), DIPEA (3.0 equiv) and corresponding aromatic amine **8a-d** (1.1 equiv) in DCM was stirred at room temperature for 2 h. Then it was extracted with DCM and the organic layer was washed with 1N HCl (aq), dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified by column chromatography.

Compound 9a

Starting from 36 mg **8a**, compound **9a** (49 mg, 82%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (br, 1H), 8.04 (d, *J* = 7.7 Hz, 2H), 7.53 – 7.30 (m, 7H), 6.66 (s, 1H), 6.49 (s, 1H), 6.32 (s, 2H), 5.94 (d, *J* = 11.4 Hz, 2H), 5.36 (s, 2H), 4.67 – 4.42 (m, 6H), 3.91 (t, *J* = 9.1 Hz, 1H), 3.57 (s, 6H), 3.11 – 3.01 (m, 1H), 2.80 – 2.66 (m, 3H); MS (ESI+) m/z = 666.1 ($M + H^+$).

Compound 9b

Starting from 36 mg **8b**, compound **9b** (60 mg, 99%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (br, 1H), 8.06 – 7.91 (m, 2H), 7.56 – 7.30 (m, 7H), 6.66 (s, 1H), 6.49 (s, 1H), 6.31 (s, 2H), 5.94 (d, *J* = 9.7 Hz, 2H), 5.37 (s, 2H), 4.62 – 4.50 (m, 5H), 4.45 (t, *J* = 7.2 Hz, 1H), 3.92 (t, *J* = 9.0 Hz, 1H), 3.55 (s, 6H), 3.12 – 3.01 (m, 1H), 2.80 – 2.64 (m, 3H); MS (ESI+) m/z = 666.2 ($M + H^+$).

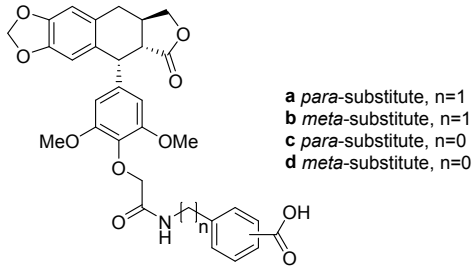
Compound 9c

Starting from 35 mg **8c**, compound **9c** (45 mg, 77%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.54 – 7.30 (m, 5H), 6.67 (s, 1H), 6.49 (s, 1H), 6.40 (s, 2H), 5.94 (d, *J* = 10.4 Hz, 2H), 5.35 (s, 2H), 4.68 – 4.56 (m, 3H), 4.46 (t, *J* = 7.2 Hz, 1H), 3.93 (t, *J* = 9.0 Hz, 1H), 3.80 (s, 6H), 3.14 – 2.99 (m, 1H), 2.86 – 2.60 (m, 3H); MS (ESI+) m/z = 652.1 ($M + H^+$).

Compound 9d

Starting from 35 mg **8d**, compound **9d** (54 mg, 92%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.99 (s, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.53 – 7.30 (m, 6H), 6.68 (s, 1H), 6.49 (s, 1H), 6.38 (s, 2H), 5.95 (d, *J* = 11.2 Hz, 2H), 5.35 (s, 2H), 4.75 – 4.53 (m, 3H), 4.46 (t, *J* = 7.2 Hz, 1H), 3.92 (t, *J* = 8.8 Hz, 1H), 3.76 (s, 6H), 3.23 – 3.02 (m, 1H), 2.85 – 2.61

(m, 3H); MS (ESI+) m/z = 652.2 ($M + H^+$).



10a-d

Representative procedure for 10a-d

A mixture of compounds **9a-d** and 20% $Pd(OH)_2$ (20% w/w) in methanol-ethyl acetate (3:1 v/v) was hydrogenated at room temperature for 12 h. The mixture was filtered and the filtrate was evaporated to dryness to give the corresponding compound, which was used directly in the next step.

Compound 10a

Starting from 49 mg **9a** and 5 mg 20 % $Pd(OH)_2$, compound **10a** (42 mg, 99%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (br, 1H), 8.07 (d, *J* = 7.5 Hz, 2H), 7.40 (d, *J* = 7.6 Hz, 2H), 6.67 (s, 1H), 6.50 (s, 1H), 6.33 (s, 2H), 5.95 (d, *J* = 13.0 Hz, 2H), 4.68 – 4.52 (m, 5H), 4.48 (t, *J* = 6.9 Hz, 1H), 3.93 (t, *J* = 8.9 Hz, 1H), 3.57 (s, 6H), 3.08 (d, *J* = 14.7 Hz, 1H), 2.83 – 2.66 (m, 3H); MS (ESI-) m/z = 574.1 ($M - H^+$).

Compound 10b

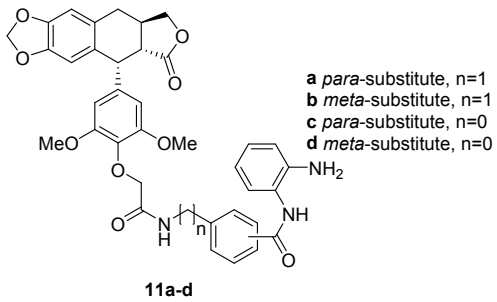
Starting from 60 mg **9b** and 6 mg 20 % $Pd(OH)_2$, compound **10b** (52 mg, 99%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (br, 1H), 8.09 – 7.98 (m, 2H), 7.61 – 7.41 (m, 2H), 6.66 (s, 1H), 6.49 (s, 1H), 6.32 (s, 2H), 5.94 (d, *J* = 12.6 Hz, 2H), 4.66 – 4.54 (m, 5H), 4.46 (t, *J* = 6.5 Hz, 1H), 3.92 (t, *J* = 8.7 Hz, 1H), 3.57 (s, 6H), 3.07 (d, *J* = 14.7 Hz, 1H), 2.84 – 2.67 (m, 3H); MS (ESI+) m/z = 576.1 ($M + H^+$).

Compound 10c

Starting from 45 mg **9c** and 5 mg 20 % $Pd(OH)_2$, compound **10c** (36 mg, 93%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 6.68 (s, 1H), 6.50 (s, 1H), 6.41 (s, 2H), 5.95 (d, *J* = 10.3 Hz, 2H), 4.77 – 4.57 (m, 3H), 4.46 (t, *J* = 6.9 Hz, 1H), 3.93 (t, *J* = 8.9 Hz, 1H), 3.81 (s, 6H), 3.14 – 3.02 (m, 1H), 2.93 – 2.56 (m, 3H); MS (ESI-) m/z = 560.1 ($M - H^+$).

Compound 10d

Starting from 54 mg **9d** and 5 mg 20 % $Pd(OH)_2$, compound **10d** (42 mg, 90%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 6.68 (s, 1H), 6.50 (s, 1H), 6.42 (s, 2H), 5.94 (d, *J* = 9.9 Hz, 2H), 4.75 – 4.56 (m, 3H), 4.55 – 4.42 (m, 1H), 3.93 (t, *J* = 8.2 Hz, 1H), 3.84 (s, 6H), 3.20 – 3.02 (m, 1H), 2.88 – 2.58 (m, 3H); MS (ESI+) m/z = 562.1 ($M + H^+$).



11a-d

Representative procedure for 11a-d

A mixture of **11a-d** (1.0 equiv), 1,2-benzenediamine (3.0 equiv), HATU (1.05 equiv), DIPEA (3.0 equiv) in DCM was stirred at room temperature for 12 h. Then it was extracted with DCM and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified by column chromatography.

Compound 11a

Starting from 45 mg **10a**, compound **11a** (19 mg, 36%) was obtained as a white solid according to above–mentioned general procedure. mp 142–143 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.38 (br, 1H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.32 – 7.26 (m, 3H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.88 – 6.77 (m, 2H), 6.68 (s, 1H), 6.49 (s, 1H), 6.28 (s, 2H), 5.94 (d, *J* = 14.1 Hz, 2H), 4.85 (dd, *J* = 15.1, 7.5 Hz, 1H), 4.77 – 4.55 (m, 3H), 4.52 (t, *J* = 7.4 Hz, 1H), 4.33 – 4.24 (m, 1H), 3.95 (t, *J* = 9.0 Hz, 1H), 3.48 (s, 6H), 3.15 – 3.05 (m, 1H), 2.83 – 2.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.46, 170.18, 165.61, 151.11, 147.21, 146.86, 142.23, 141.14, 137.02, 135.27, 133.38, 130.09, 128.33, 127.94, 127.72, 127.18, 125.46, 124.61, 119.40, 118.25, 110.31, 108.59, 107.93, 101.27, 72.35, 72.24, 55.66, 47.56, 43.71, 42.35, 33.03, 32.79; HPLC: room temperature; t_R = 10.96 min, UV_{254} = 95.2%; HRMS (ESI): m/z calcd for $C_{37}H_{35}N_3O_9Na^+$ (M + Na^+): 688.2266, found: 688.2289.

Compound 11b

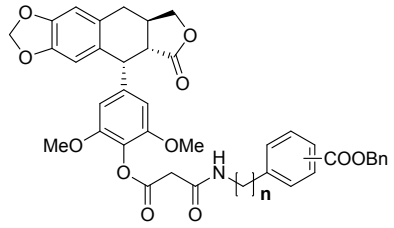
Starting from 56 mg **10b**, compound **11b** (18 mg, 28%) was obtained as a white solid according to above–mentioned general procedure. mp 136–137 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (br, 1H), 8.12 (s, 1H), 7.90 – 7.75 (m, 2H), 7.53 – 7.39 (m, 2H), 7.36 – 7.29 (m, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 7.4 Hz, 2H), 6.65 (s, 1H), 6.47 (s, 1H), 6.31 (s, 2H), 5.94 (d, *J* = 9.3 Hz, 2H), 4.64 – 4.46 (m, 5H), 4.42 (t, *J* = 6.9 Hz, 1H), 4.15 – 3.69 (m, 3H), 3.58 (s, 6H), 3.10 – 3.00 (m, 1H), 2.82 – 2.59 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.90, 170.24, 165.61, 151.31, 147.13, 146.80, 140.80, 138.98, 137.09, 135.67, 134.74, 131.34, 130.27, 129.13, 128.34, 127.25, 126.76, 126.48, 125.31, 124.55, 119.69, 118.36, 110.38, 108.54, 108.03, 101.23, 72.70, 72.07, 55.96, 47.41, 43.69, 42.75, 33.04, 32.78; HPLC: room temperature; t_R = 11.04 min, UV_{254} = 97.5%; HRMS (ESI): m/z calcd for $C_{37}H_{35}N_3O_9Na^+$ (M + Na^+): 688.2266, found: 688.2257.

Compound 11c

Starting from 36 mg **10c**, compound **11c** (12 mg, 29%) was obtained as a white solid according to above–mentioned general procedure. mp 163–164 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.97 – 7.87 (m, 3H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.39 – 7.33 (m, 1H), 7.16 – 7.08 (m, 1H), 6.91 – 6.83 (m, 2H), 6.70 (s, 1H), 6.53 (s, 1H), 6.43 (s, 2H), 6.00 – 5.94 (m, 2H), 4.68 – 4.61 (m, 3H), 4.49 (dd, *J* = 8.5, 6.6 Hz, 1H), 3.99 – 3.90 (m, 3H), 3.83 (s, 6H), 3.11 (dd, *J* = 15.1, 4.6 Hz, 1H), 2.86 – 2.73 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.86, 168.72, 165.17, 151.31, 147.20, 146.87, 141.01, 140.76, 137.39, 135.78, 130.19, 129.60, 128.53, 128.34, 127.16, 125.32, 125.28, 124.68, 119.74, 119.25, 118.36, 110.38, 108.57, 108.26, 101.27, 72.97, 72.05, 56.34, 47.42, 43.74, 33.08, 32.85; HPLC: room temperature; t_R = 11.42 min, UV_{254} = 95.0%; HRMS (ESI): m/z calcd for $C_{36}H_{33}N_3O_9Na^+$ (M + Na^+): 674.2109, found: 674.2084.

Compound 11d

Starting from 42 mg **10d**, compound **11d** (17 mg, 35%) was obtained as a white solid according to above–mentioned general procedure. mp 151–152 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.15 (s, 1H), 8.00 (s, 1H), 7.92 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.13 – 7.06 (m, 1H), 6.89 – 6.81 (m, 2H), 6.68 (s, 1H), 6.50 (s, 1H), 6.41 (s, 2H), 5.95 (dd, *J* = 9.8, 1.3 Hz, 2H), 4.67 – 4.59 (m, 3H), 4.46 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.98 – 3.85 (m, 3H), 3.82 (s, 6H), 3.09 (dd, *J* = 14.9, 4.4 Hz, 1H), 2.84 – 2.63 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.88, 168.74, 165.39, 151.28, 147.19, 146.86, 140.78, 138.21, 137.34, 135.72, 135.03, 130.20, 129.51, 128.33, 127.25, 125.39, 124.45, 122.94, 122.88, 119.68, 118.60, 118.28, 110.36, 108.57, 108.22, 101.26, 72.89, 72.06, 56.35, 47.40, 43.73, 33.06, 32.84; HPLC: room temperature; t_R = 11.58 min, UV_{254} = 95.8%; HRMS (ESI): m/z calcd for $C_{36}H_{33}N_3O_9Na^+$ (M + Na^+): 674.2109, found: 674.2083.



12a-d

a *para*-substitute, n=1
b *meta*-substitute, n=1
c *para*-substitute, n=0
d *meta*-substitute, n=0

Representative procedure for 12a-d

A mixture of compound **4** (1.0 equiv), HATU (1.05 equiv), DIPEA (3.0 equiv) and corresponding aromatic amine **8a-d** (1.1 equiv) in DCM was stirred at room temperature for 2 h. Then it was extracted with DCM and the organic layer was washed with 1N HCl (aq), dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified by column chromatography.

Compound 12a

Starting from 30 mg **8a**, compound **12a** (46 mg, 87%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.3 Hz, 2H), 7.62 (t, *J* = 5.5 Hz, 1H), 7.50 – 7.31 (m, 7H), 6.66 (s, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 5.94 (dd, *J* = 6.8, 1.2 Hz, 2H), 5.35 (s, 2H), 4.62 (d, *J* = 4.8 Hz, 1H), 4.57 (d, *J* = 5.8 Hz, 2H), 4.45 (dd, *J* = 8.5, 6.8 Hz, 1H), 3.92 (t, *J* = 9.3 Hz, 1H), 3.69 (s, 2H), 3.60 (s, 6H), 3.07 (dd, *J* = 15.7, 4.8 Hz, 1H), 2.79 – 2.61 (m, 3H); MS (ESI+) m/z = 694.3 (M + H^+).

Compound 12b

Starting from 30 mg **8b**, compound **12b** (37 mg, 70%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.94 (m, 2H), 7.67 – 7.49 (m, 2H), 7.47 – 7.31 (m, 6H), 6.66 (s, 1H), 6.49 (s, 1H), 6.37 (s, 2H), 5.94 (d, *J* = 7.2 Hz, 2H), 5.35 (s, 2H), 4.65 – 4.59 (m, 1H), 4.55 (d, *J* = 5.2 Hz, 2H), 4.44 (t, *J* = 7.5 Hz, 1H), 3.92 (t, *J* = 9.2 Hz, 1H), 3.68 (s, 2H), 3.58 (s, 6H), 3.13 – 3.00 (m, 1H), 2.85 – 2.60 (m, 3H); MS (ESI+) m/z = 694.3 (M + H^+).

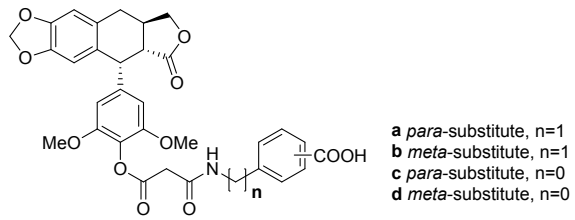
Compound 12c

Starting from 29 mg **8c**, compound **12c** (49 mg, 95%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.04 (d, *J* = 8.1 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.48 – 7.30 (m, 5H), 6.68 (s, 1H), 6.52 (s, 1H), 6.43 (s, 2H), 5.95 (d, *J* = 8.4 Hz, 2H), 5.34 (s, 2H), 4.65 (d, *J* = 3.8 Hz, 1H), 4.47 (t, *J* = 7.5 Hz, 1H), 3.94 (t, *J* = 9.2 Hz, 1H), 3.80 (s, 2H), 3.70 (s, 6H), 3.14 – 3.04 (m, 1H), 2.86 – 2.64 (m, 3H); MS (ESI+) m/z = 680.2(M + H^+).

Compound 12d

Starting from 29 mg **8d**, compound **12d** (51 mg, 99%) was obtained as a colorless oil according to above–mentioned general procedure.^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.08 – 7.95 (m, 2H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.51 – 7.30 (m, 6H), 6.68 (s, 1H), 6.53 (s, 1H), 6.42 (s, 2H), 5.95 (d, *J* = 8.2 Hz, 2H), 5.35 (s, 2H), 4.65 (d, *J* = 3.6 Hz,

1H), 4.48 (t, *J* = 7.5 Hz, 1H), 3.94 (t, *J* = 9.2 Hz, 1H), 3.79 (s, 2H), 3.69 (s, 6H), 3.13 – 3.05 (m, 1H), 2.84 – 2.67 (m, 3H); MS (ESI+) m/z = 680.0 ($M + H^+$).



13a-d

Representative procedure for 13a-d

A mixture of compounds **12a-d** and 20% $Pd(OH)_2$ (20% w/w) in methanol-ethyl acetate (3:1 v/v) was hydrogenated at room temperature for 12 h. The mixture was filtered and the filtrate was evaporated to dryness to give the corresponding compound, which was used directly in the next step.

Compound 13a

Starting from 42 mg **12a** and 4 mg 20 % $Pd(OH)_2$, compound **13a** (36 mg, 98%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.2 Hz, 2H), 7.68 (t, *J* = 5.4 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 2H), 6.66 (s, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 5.94 (d, *J* = 7.2 Hz, 2H), 4.63 (d, *J* = 4.5 Hz, 1H), 4.60 (d, *J* = 5.6 Hz, 2H), 4.51 – 4.43 (m, 1H), 3.92 (t, *J* = 9.2 Hz, 1H), 3.71 (s, 2H), 3.61 (s, 6H), 3.07 (dd, *J* = 15.8, 4.4 Hz, 1H), 2.83 – 2.67 (m, 3H); MS (ESI-) m/z = 602.0 ($M - H^+$).

Compound 13b

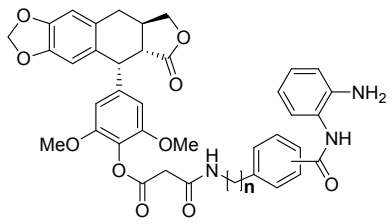
Starting from 37 mg **12b** and 4 mg 20 % $Pd(OH)_2$, compound **13b** (32 mg, 99%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 – 7.94 (m, 2H), 7.77 – 7.66 (m, 1H), 7.62 – 7.52 (m, 1H), 7.49 – 7.39 (m, 1H), 6.66 (s, 1H), 6.49 (s, 1H), 6.37 (s, 2H), 5.93 (d, *J* = 5.9 Hz, 2H), 4.75 – 4.52 (m, 3H), 4.51 – 4.38 (m, 1H), 3.98 – 3.84 (m, 1H), 3.72 (s, 2H), 3.61 (s, 6H), 3.16 – 2.94 (m, 1H), 2.86 – 2.56 (m, 3H); MS (ESI-) m/z = 602.0 ($M - H^+$).

Compound 13c

Starting from 49 mg **12c** and 5 mg 20 % $Pd(OH)_2$, compound **13c** (30 mg, 71%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 2H), 7.67 (d, *J* = 7.8 Hz, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 6.43 (s, 2H), 5.96 (d, *J* = 8.5 Hz, 2H), 4.66 (s, 1H), 4.56 – 4.43 (m, 1H), 3.95 (t, *J* = 8.6 Hz, 1H), 3.82 (s, 2H), 3.71 (s, 6H), 3.22 – 3.00 (m, 1H), 2.93 – 2.57 (m, 3H); MS (ESI+) m/z = 589.9 ($M + H^+$).

Compound 13d

Starting from 54 mg **12d** and 5 mg 20 % $Pd(OH)_2$, compound **13d** (30 mg, 64%) was obtained as a colorless oil according to above–mentioned general procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.10 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 6.68 (s, 1H), 6.53 (s, 1H), 6.44 (s, 2H), 5.95 (d, *J* = 8.5 Hz, 2H), 4.66 (s, 1H), 4.48 (t, *J* = 7.2 Hz, 1H), 3.94 (t, *J* = 8.9 Hz, 1H), 3.82 (s, 2H), 3.72 (s, 6H), 3.14 – 3.05 (m, 1H), 2.88 – 2.64 (m, 3H); MS (ESI-) m/z = 588.0 ($M - H^+$).



14a-d

a *para*-substitute, n=1
b *meta*-substitute, n=1
c *para*-substitute, n=0
d *meta*-substitute, n=0

Representative procedure for 14a-d

A mixture of **13a-d** (1.0 equiv), 1,2-benzenediamine (3.0 equiv), HATU (1.05 equiv), DIPEA (3.0 equiv) in DCM was stirred at room temperature for 12 h. Then it was extracted with DCM and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified by column chromatography.

Compound 14a

Starting from 37 mg **13a**, compound **14a** (16 mg, 38%) was obtained as a white solid according to above–mentioned general procedure. mp 149–151 ^oC, ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.86 (d, *J* = 7.9 Hz, 2H), 7.63 (m, 1H), 7.41 (d, *J* = 7.9 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.91 – 6.79 (m, 2H), 6.66 (s, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 5.95 (s, 1H), 5.93 (s, 1H), 4.62 (d, *J* = 4.6 Hz, 1H), 4.59 (d, *J* = 5.7 Hz, 2H), 4.49 – 4.42 (m, 1H), 3.92 (t, *J* = 9.2 Hz, 1H), 3.69 (s, 2H), 3.61 (s, 6H), 3.07 (dd, *J* = 15.6, 4.4 Hz, 1H), 2.82 – 2.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.85, 166.65, 164.76, 163.79, 151.04, 147.22, 146.84, 142.09, 140.65, 139.84, 133.38, 130.01, 128.35, 127.79, 127.74, 127.21, 126.80, 125.21, 124.63, 119.79, 118.42, 110.46, 108.57, 107.65, 101.27, 72.08, 56.10, 47.41, 43.84, 43.29, 41.46, 33.06, 32.81; HPLC: room temperature; t_R = 10.11 min, UV_{254} = 95.4%; HRMS (ESI): m/z calcd for $C_{38}H_{35}N_3O_{10}Na^+$ ($M + Na^+$): 716.2215, found: 716.2228.

Compound 14b

Starting from 32 mg **13b**, compound **14b** (16 mg, 44%) was obtained as a white solid according to above–mentioned general procedure. mp 144–145 ^oC, ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.88 – 7.77 (m, 2H), 7.62 – 7.40 (m, 3H), 7.31 – 7.28 (m, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.94 – 6.74 (m, 2H), 6.67 (s, 1H), 6.49 (s, 1H), 6.37 (s, 2H), 5.94 (d, *J* = 7.6 Hz, 2H), 4.69 – 4.51 (m, 3H), 4.44 (t, *J* = 7.6 Hz, 1H), 3.99 – 3.80 (m, 3H), 3.70 (s, 2H), 3.59 (s, 6H), 3.12 – 3.01 (m, 1H), 2.83 – 2.59 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.81, 166.84, 165.49, 164.84, 151.06, 147.22, 146.83, 140.68, 139.86, 138.74, 134.72, 131.01, 130.04, 129.19, 128.36, 127.15, 126.80, 126.68, 126.14, 125.21, 124.53, 119.66, 118.32, 110.47, 108.57, 107.63, 101.27, 72.07, 56.12, 47.42, 43.82, 43.30, 41.50, 33.07, 32.77; HPLC: room temperature; t_R = 10.26 min, UV_{254} = 95.1%; HRMS (ESI): m/z calcd for $C_{38}H_{35}N_3O_{10}Na^+$ ($M + Na^+$): 716.2215, found: 716.2258.

Compound 14c

Starting from 30 mg **13c**, compound **14c** (13 mg, 38%) was obtained as a white solid according to above–mentioned general procedure. mp 166–168 ^oC, ^1H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 1H), 7.95 – 7.81 (m, 3H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 7.2 Hz, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 6.44 (s, 2H), 5.96 (d, *J* = 8.8 Hz, 2H), 4.66 (d, *J* = 3.1 Hz, 1H), 4.48 (t, *J* = 7.4 Hz, 1H), 3.95 (t, *J* = 9.2 Hz, 1H), 3.88 (br, 2H), 3.81 (s, 2H), 3.71 (s, 6H), 3.13 – 3.06 (m, 1H), 2.85 – 2.63 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.79, 167.16, 162.73, 161.61, 151.08, 147.25, 146.86, 140.88, 140.65, 140.07, 130.04, 129.90, 128.43, 128.37, 127.20, 126.76, 125.17, 124.66, 119.86, 119.76, 118.45, 110.51, 108.59, 107.72, 101.29, 72.08, 56.27, 47.44, 43.86, 41.73, 33.10, 32.82; HPLC: room temperature; t_R = 10.28 min, UV_{254} = 95.3%; HRMS (ESI): m/z calcd for $C_{37}H_{34}N_3O_{10}$ ($M + H^+$): 680.2239, found: 680.2255.

Compound 14d

Starting from 30 mg **13d**, compound **14d** (13 mg, 38%) was obtained as a white solid according to above–mentioned general procedure. mp 159–160 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.08 (s, 1H), 7.94 (s, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.09 (t, *J* = 7.7 Hz, 1H), 6.85 (d, *J* = 6.6 Hz, 2H), 6.68 (s, 1H), 6.53 (s, 1H), 6.43 (s, 2H), 5.95 (d, *J* = 8.4 Hz, 2H), 4.65 (d, *J* = 3.2 Hz, 1H), 4.47 (t, *J* = 7.3 Hz, 1H), 3.99 – 3.84 (m, 3H), 3.80 (s, 2H), 3.72 (s, 6H), 3.14 – 3.05 (m, 1H), 2.84 – 2.64 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.82, 166.83, 165.28, 162.91, 151.10, 147.24, 146.85, 140.75, 140.03, 138.04, 135.07, 130.05, 129.53, 128.38, 127.28, 126.77, 125.32, 124.43, 123.46, 119.73, 118.92, 118.34, 110.50, 108.57, 107.70, 101.28, 72.09, 56.29, 47.43, 43.86, 41.95, 33.09, 32.79; HPLC: room temperature; t_R = 10.23 min, UV_{254} = 96.6%; HRMS (ESI): m/z calcd for $C_{37}H_{34}N_3O_{10}$ (M + H^+): 680.2239, found: 680.2262.

Figure S1. ^{1}H NMR Spectrum of **3** (400 MHz, $CDCl_3$)

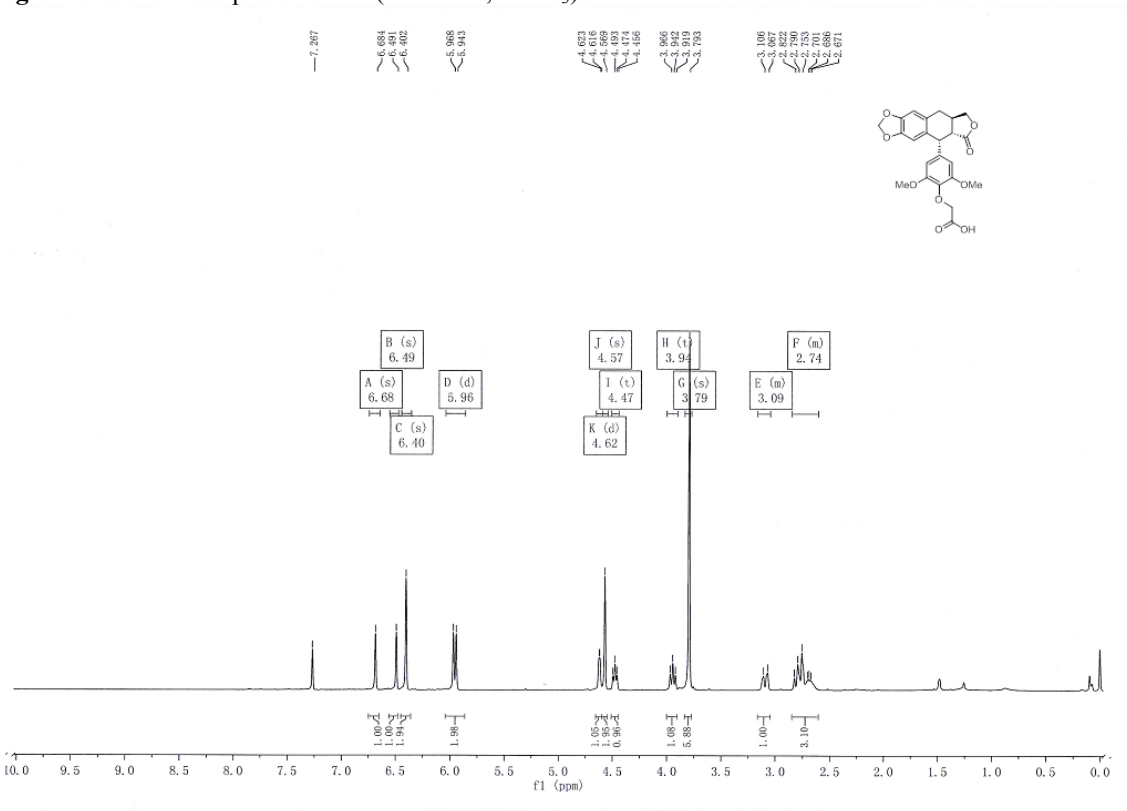


Figure S2. ^{1}H NMR Spectrum of **9a** (400 MHz, $CDCl_3$)

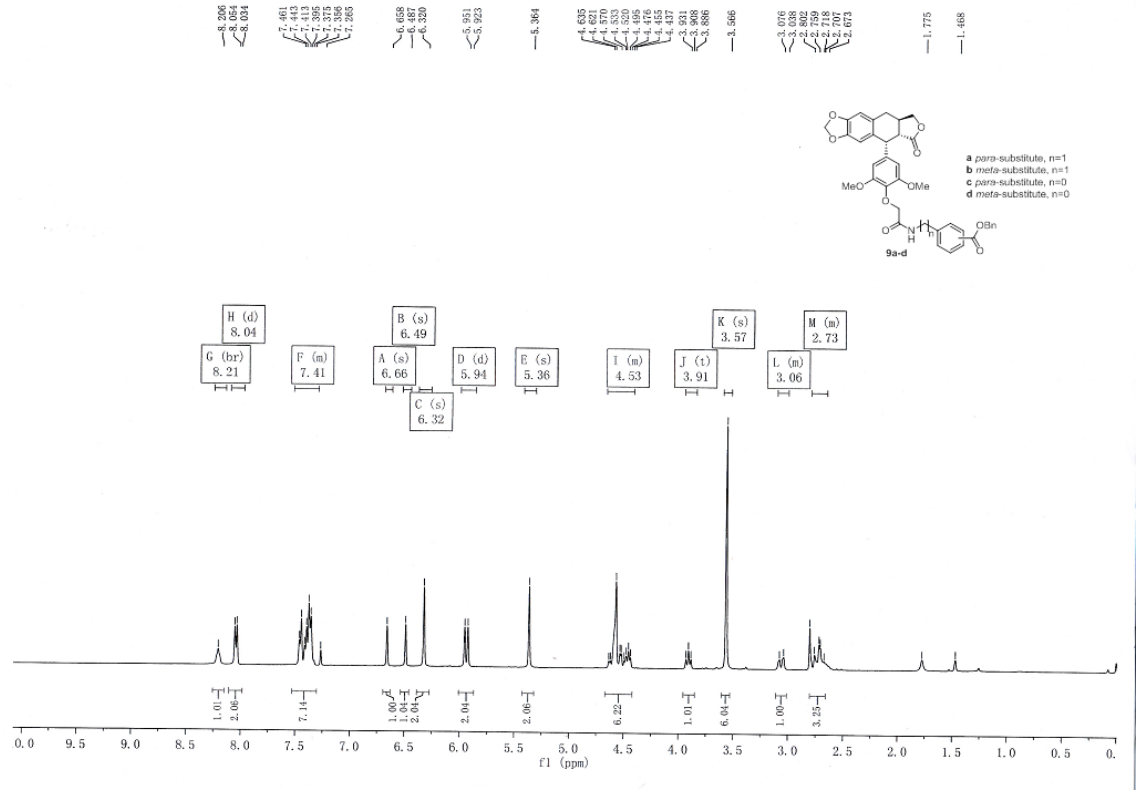


Figure S3 ^{1}H NMR Spectrum of **9b** (400 MHz, $CDCl_3$)

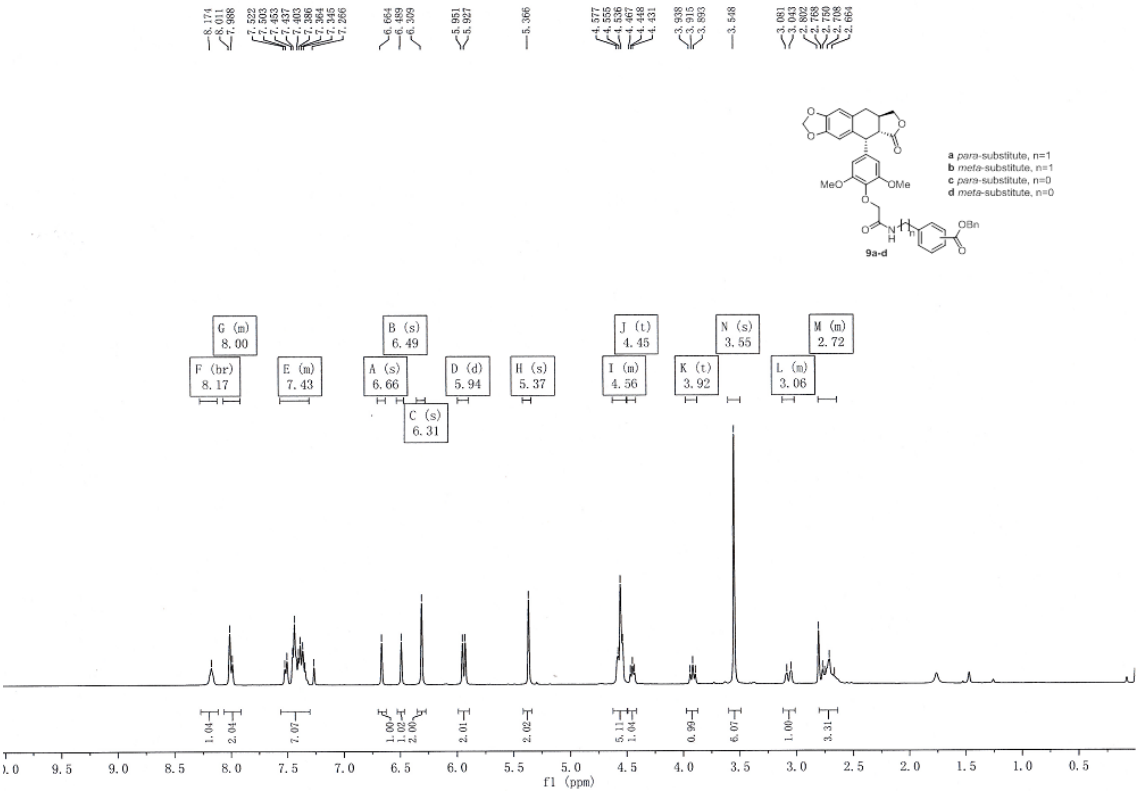


Figure S4. ^{1}H NMR Spectrum of **9c** (400 MHz, $CDCl_3$)

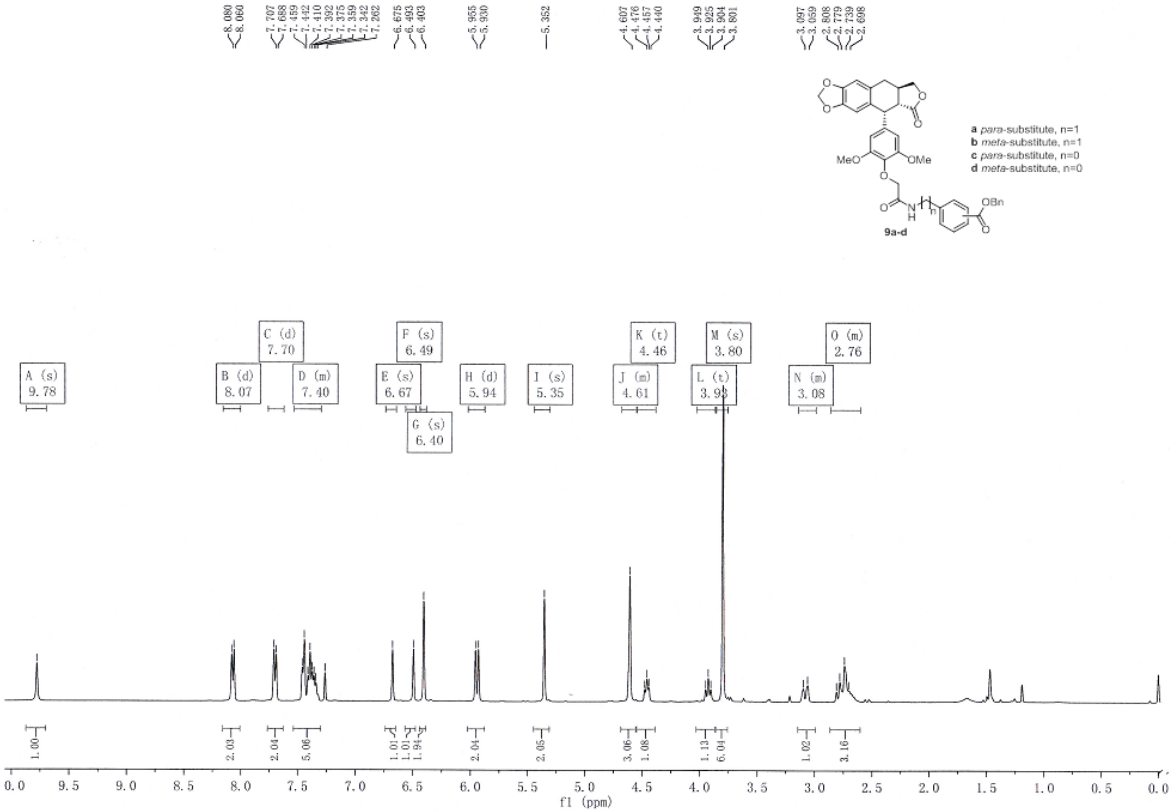


Figure S5. ^{1}H NMR Spectrum of **9d** (400 MHz, $CDCl_3$)

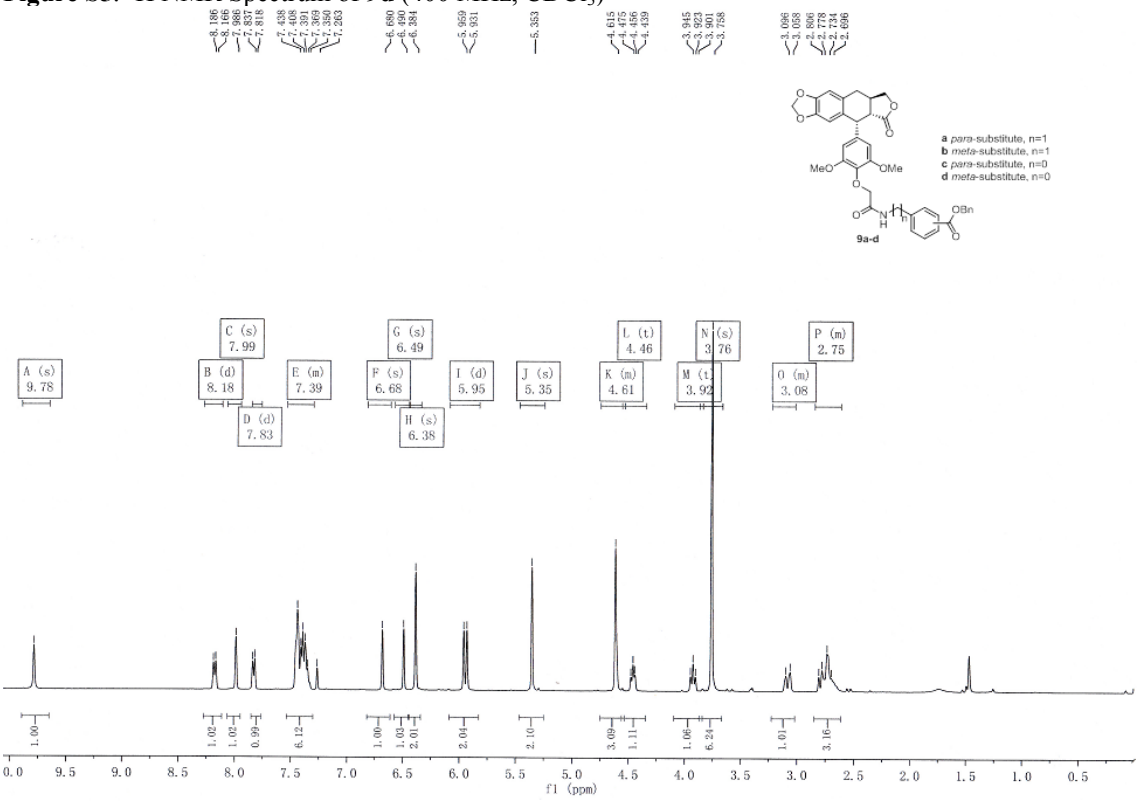


Figure S6 ^{1}H NMR Spectrum of **10a** (400 MHz, $CDCl_3$)

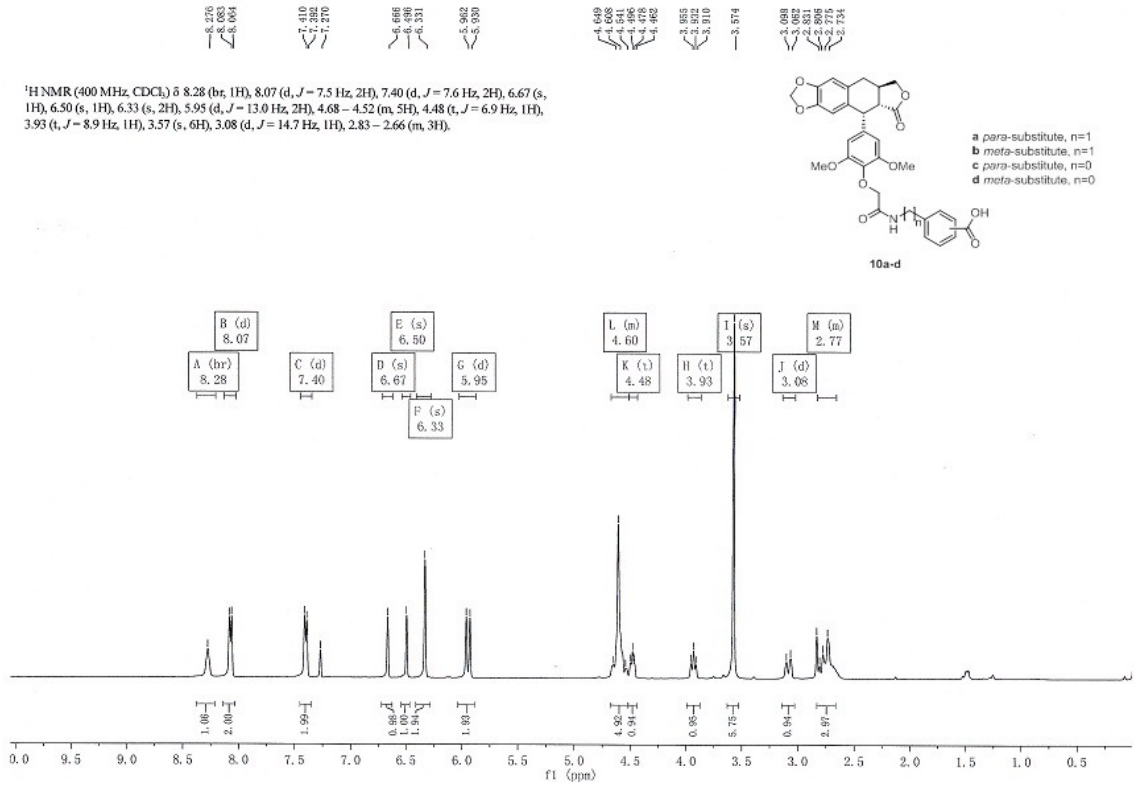


Figure S7. ^{1}H NMR Spectrum of **10b** (400 MHz, $CDCl_3$)

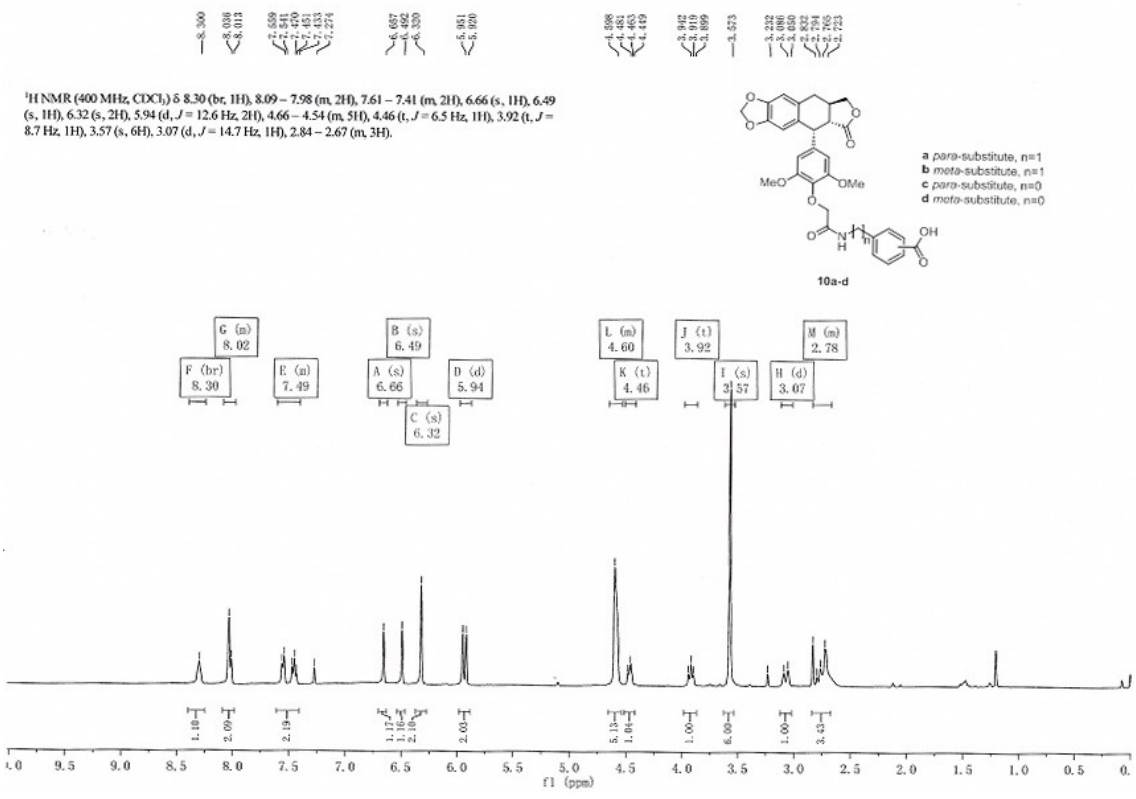


Figure S8. ^{1}H NMR Spectrum of **10c** (400 MHz, $CDCl_3$)

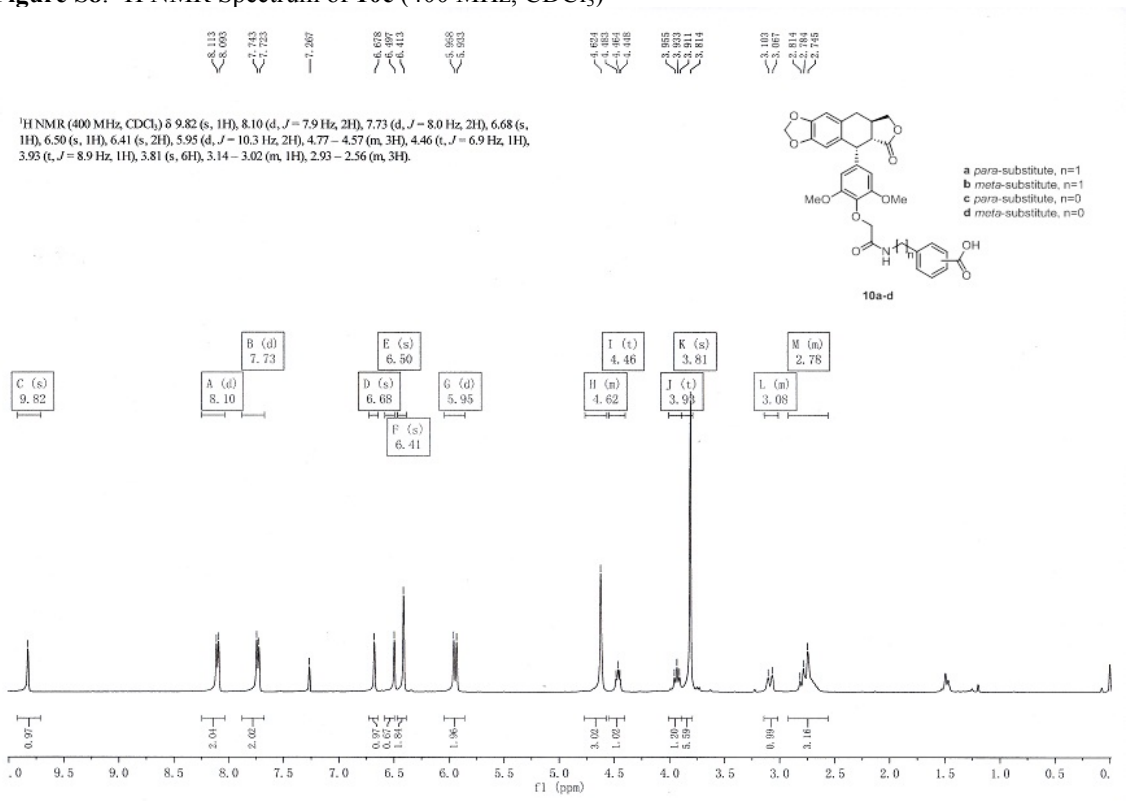


Figure S9. ^{1}H NMR Spectrum of **10d** (400 MHz, $CDCl_3$)

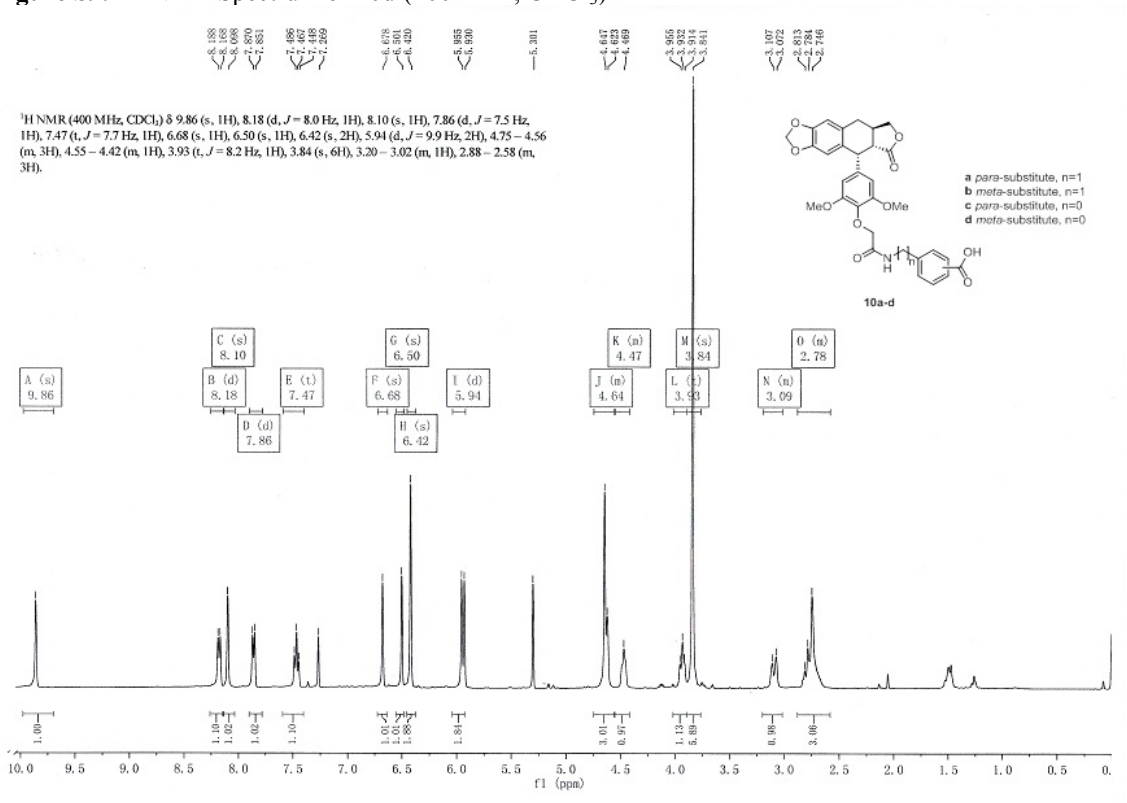


Figure S10. ^{1}H NMR Spectrum of **11a** (400 MHz, $CDCl_3$)

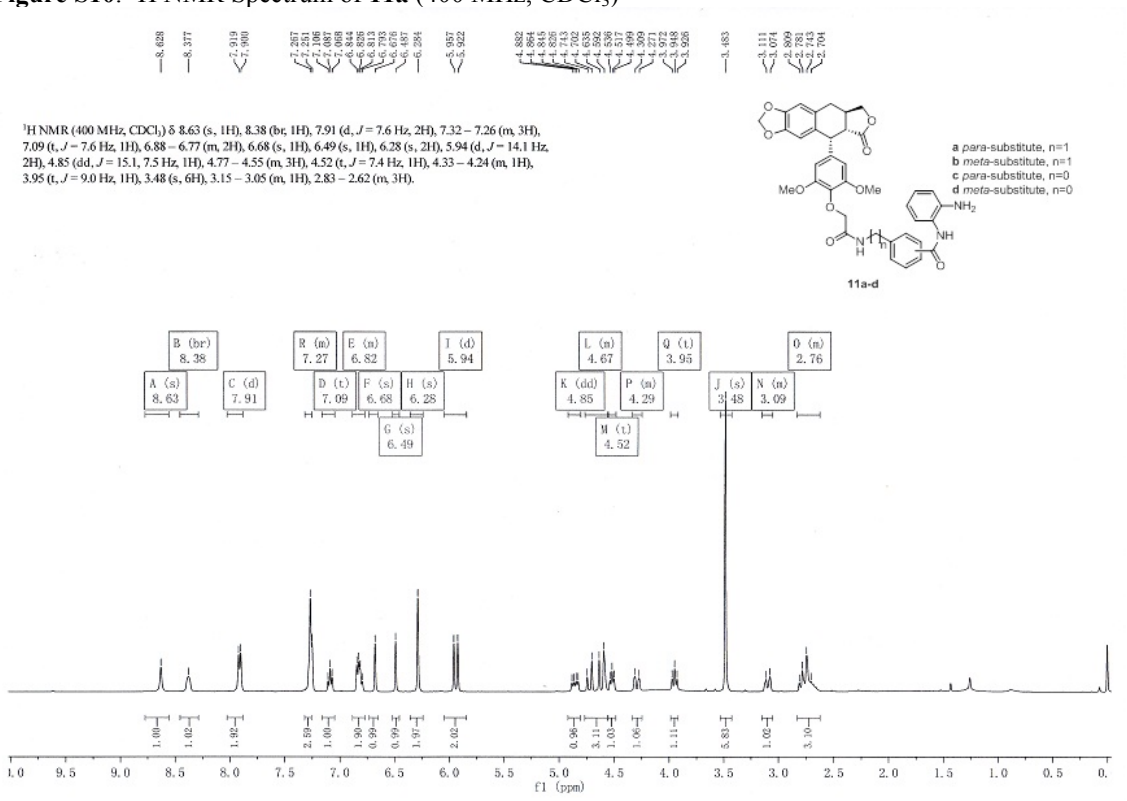


Figure S11. ^{1}H NMR Spectrum of **11b** (400 MHz, $CDCl_3$)

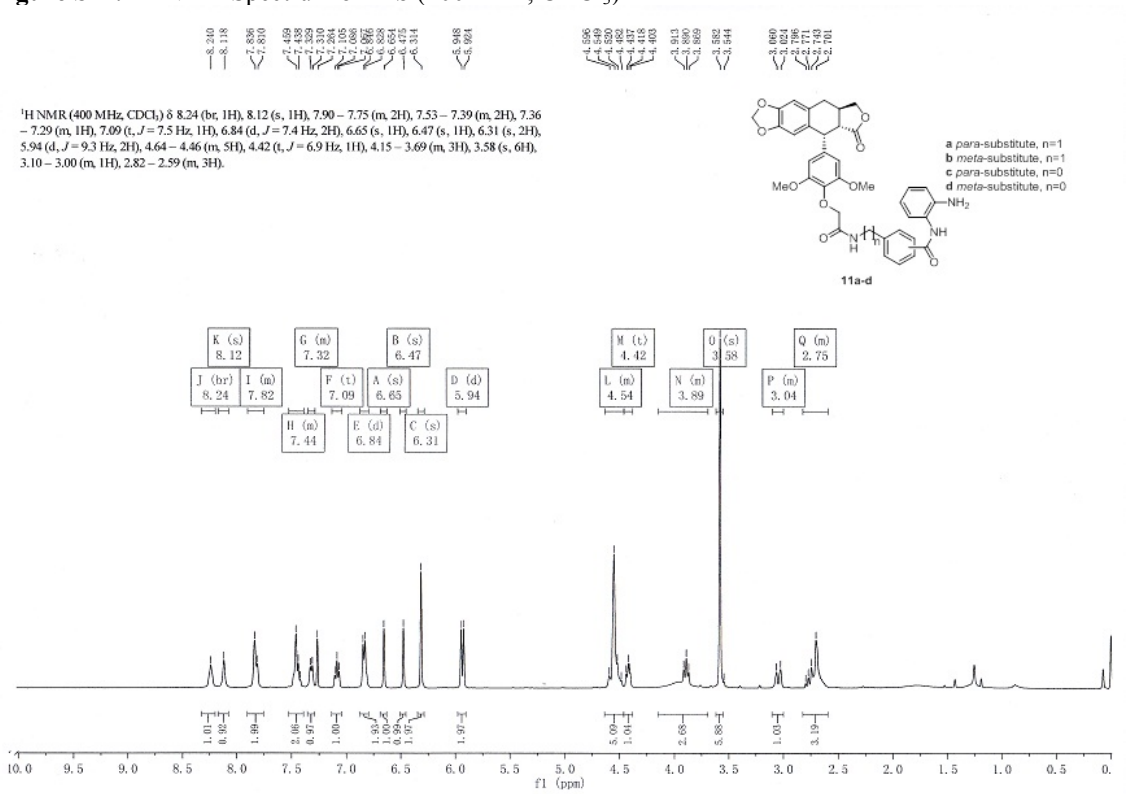


Figure S12. ^{1}H NMR Spectrum of **11c** (400 MHz, $CDCl_3$)

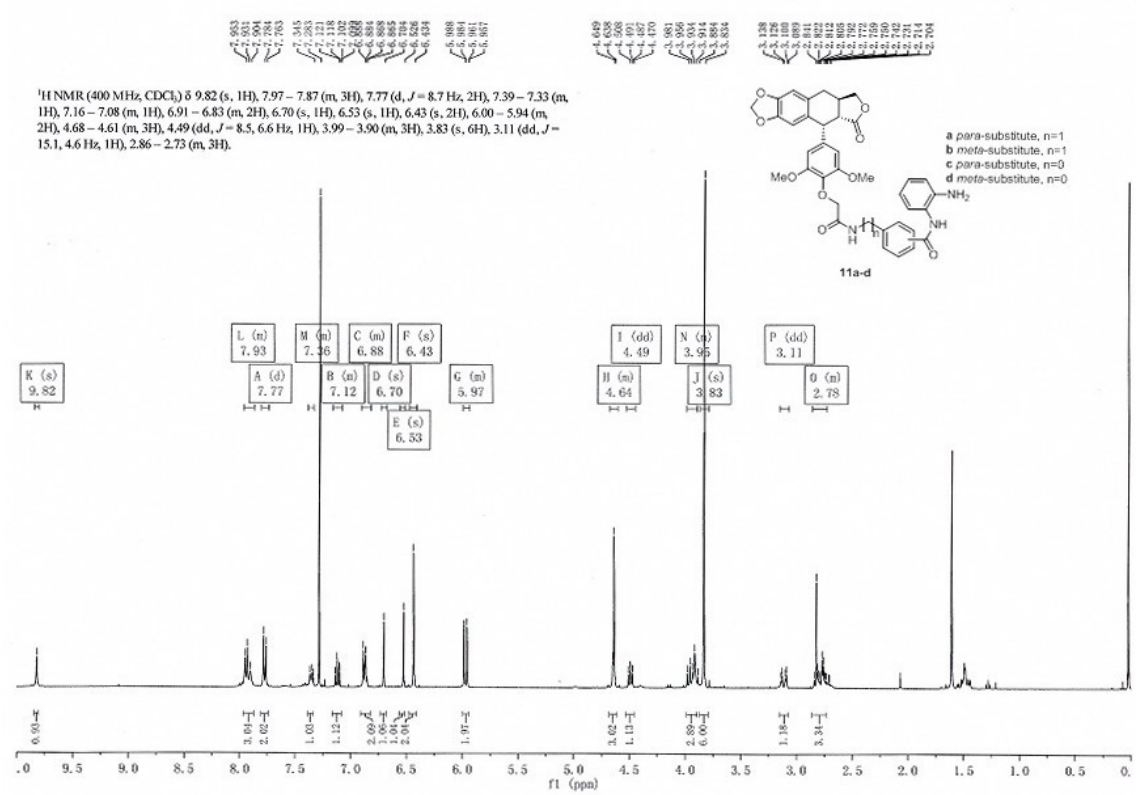


Figure S13. ^{1}H NMR Spectrum of **11d** (400 MHz, $CDCl_3$)

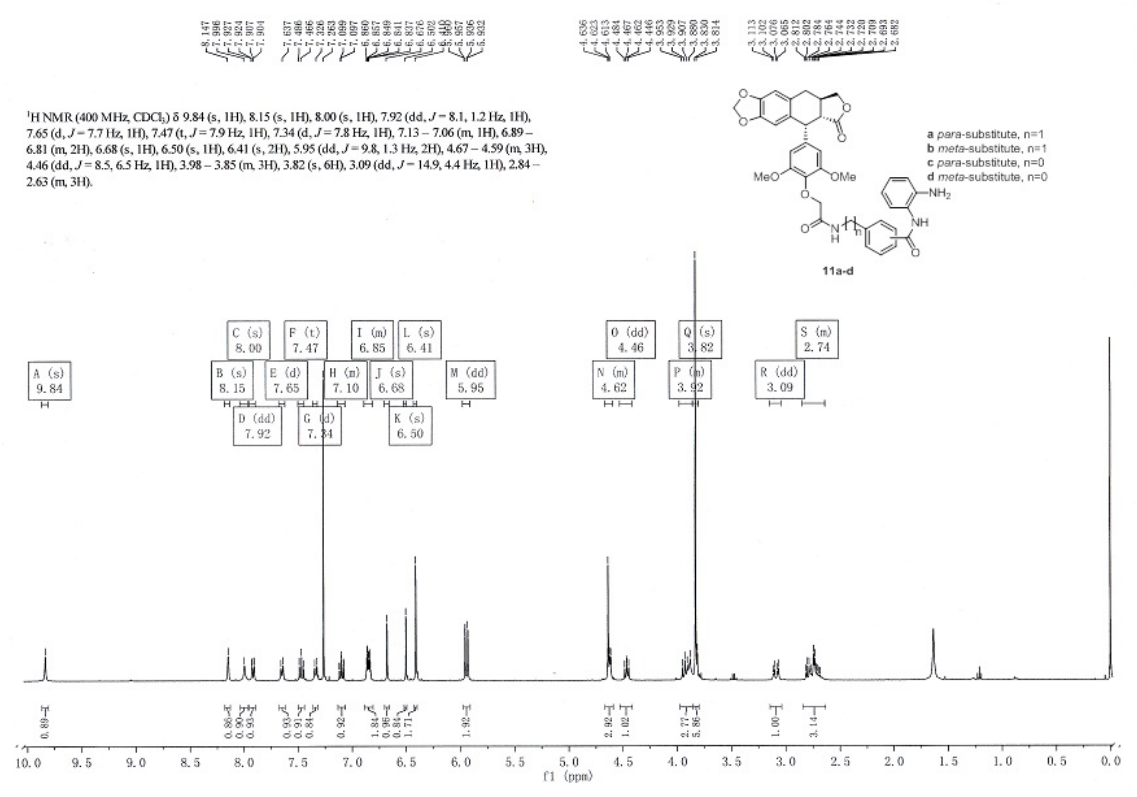


Figure S14. ^{1}H NMR Spectrum of **12a** (400 MHz, $CDCl_3$)

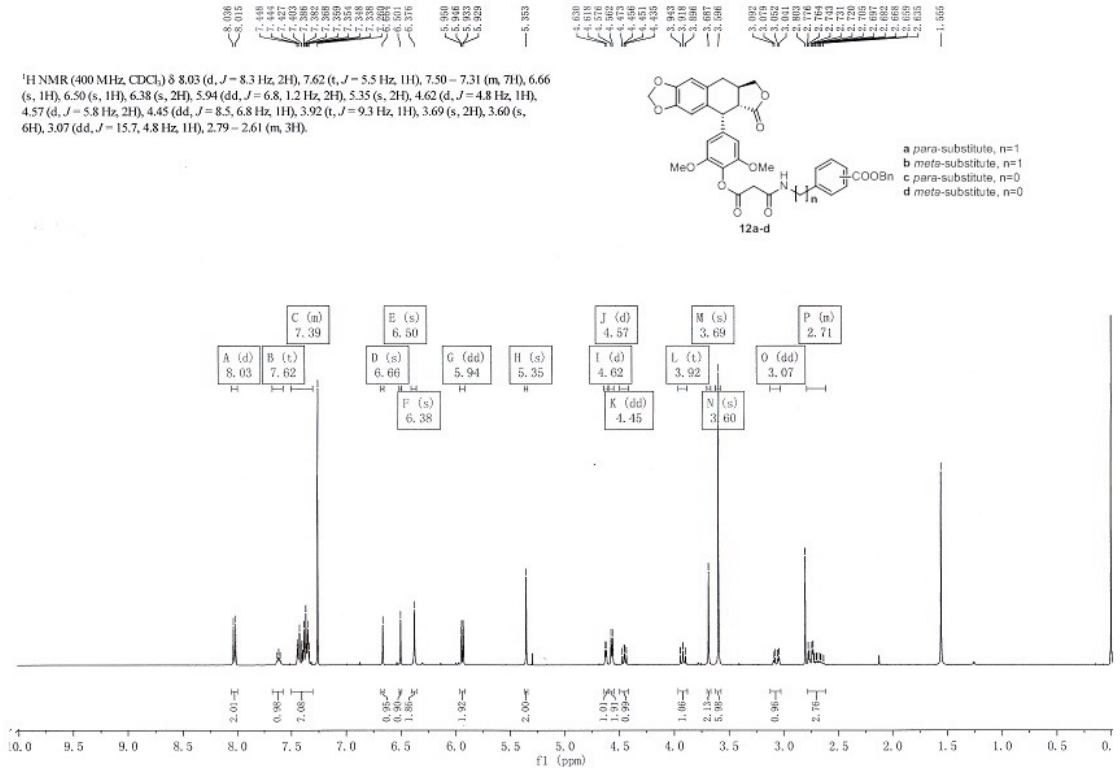


Figure S15. ^{1}H NMR Spectrum of **12b** (400 MHz, $CDCl_3$)

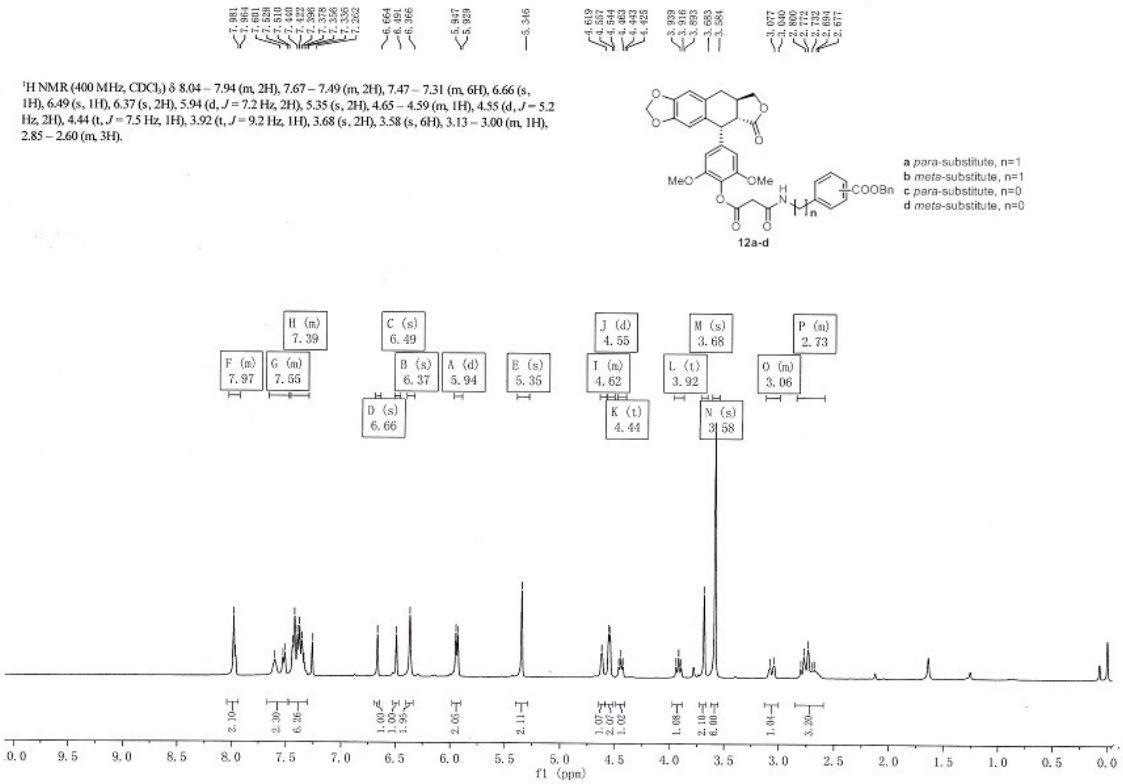


Figure S16. ^{1}H NMR Spectrum of **12c** (400 MHz, $CDCl_3$)

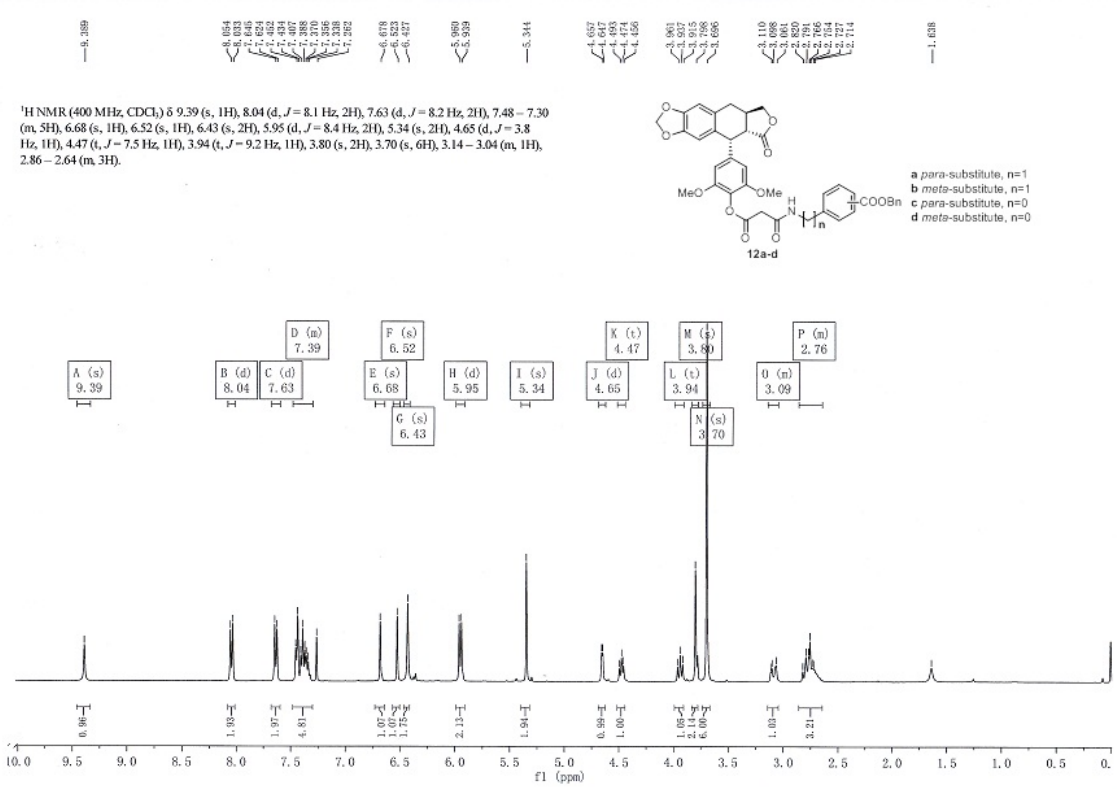


Figure S17. ^{1}H NMR Spectrum of **12d** (400 MHz, $CDCl_3$)

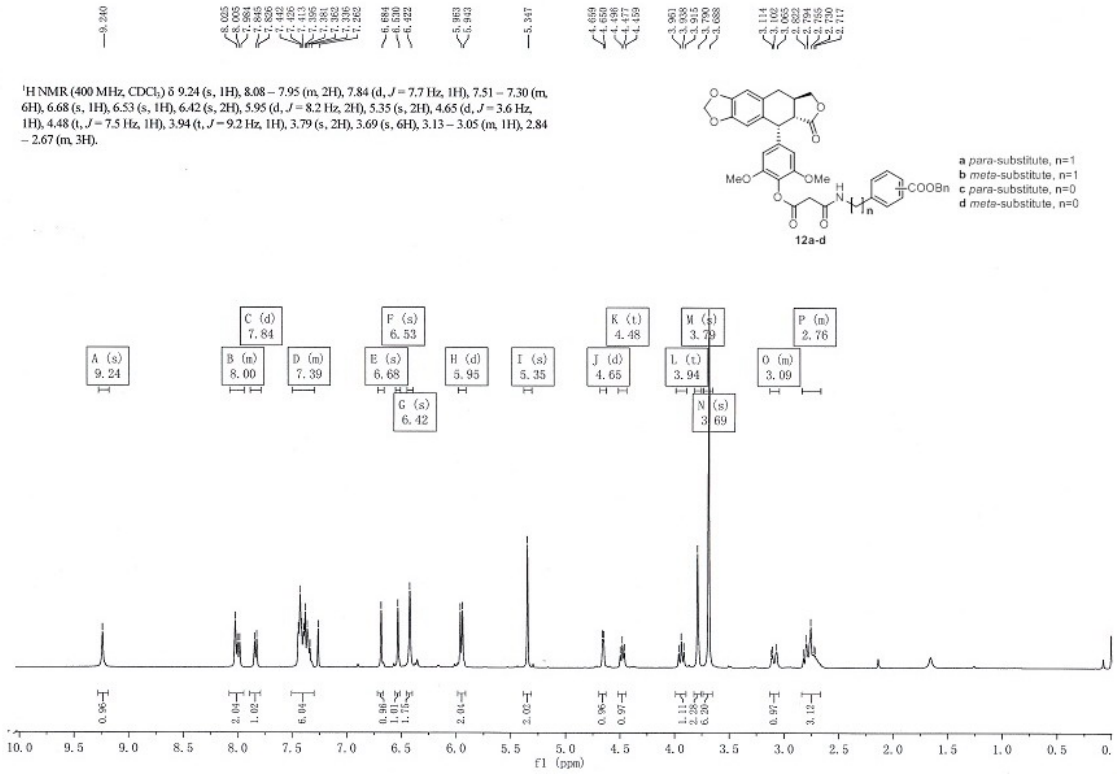


Figure S18. ^{1}H NMR Spectrum of **13a** (400 MHz, $CDCl_3$)

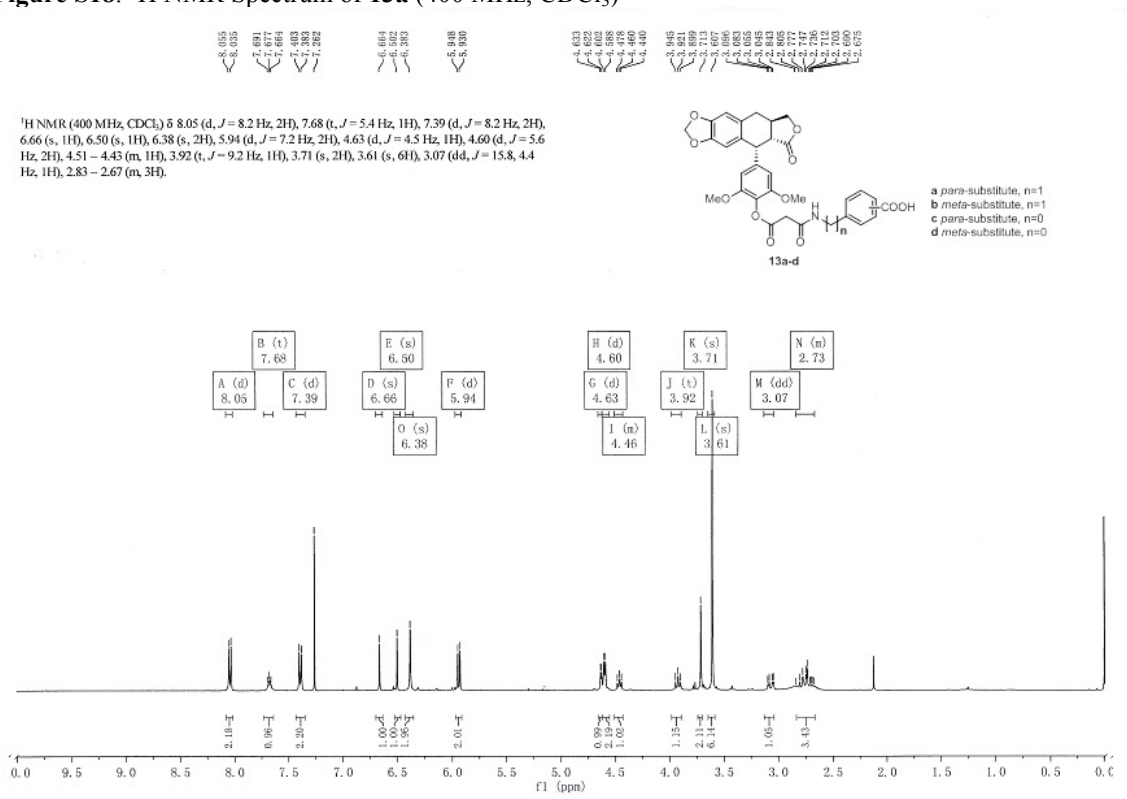


Figure S19. ^{1}H NMR Spectrum of **13b** (400 MHz, $CDCl_3$)

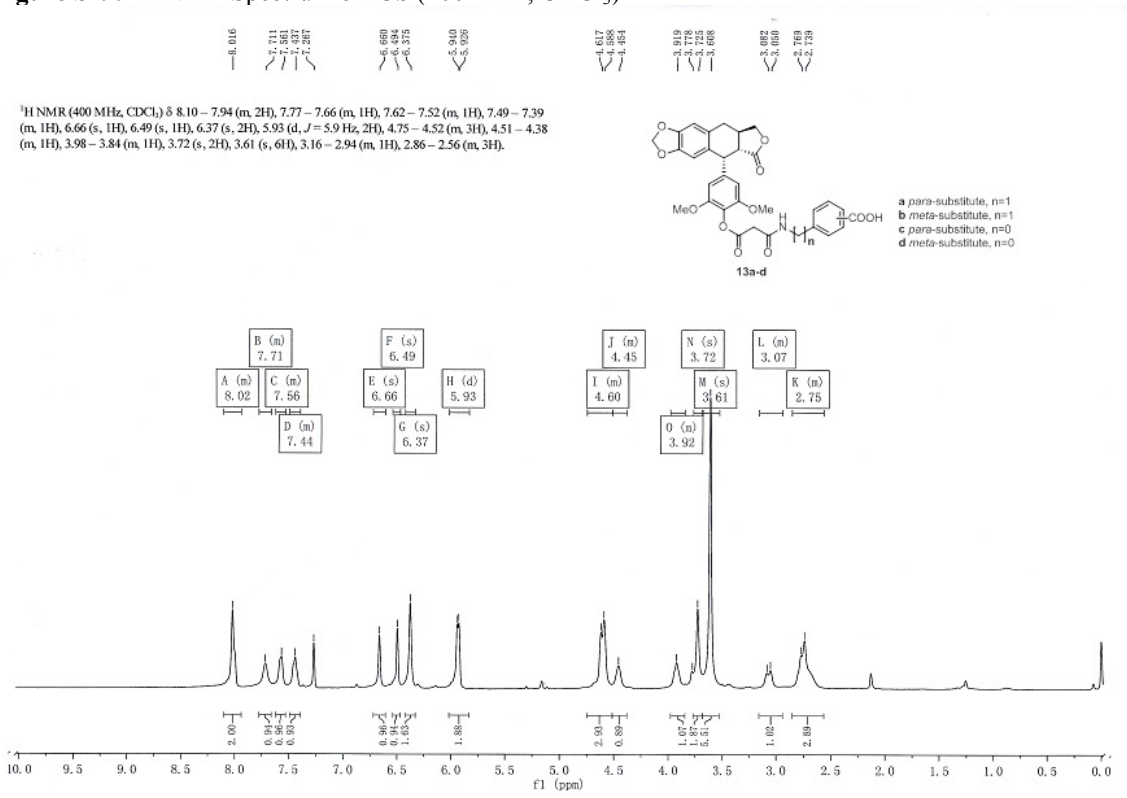


Figure S20. ^{1}H NMR Spectrum of **13c** (400 MHz, $CDCl_3$)

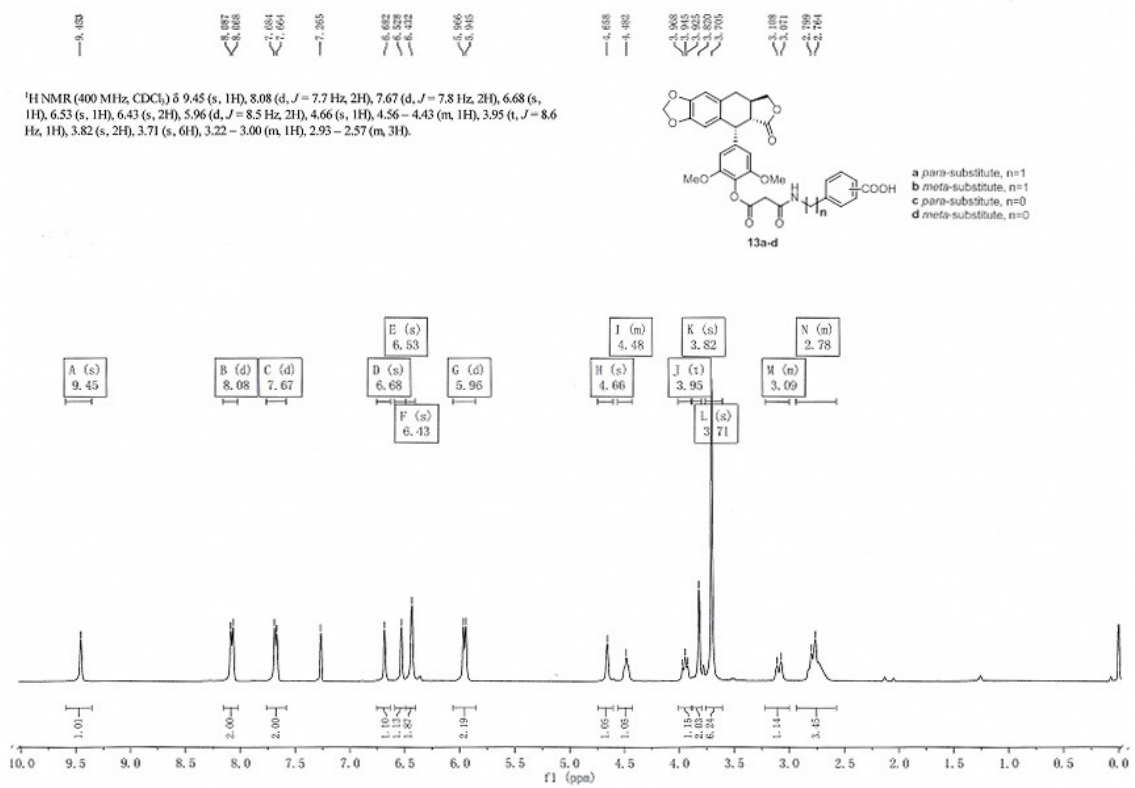


Figure S21. ^{1}H NMR Spectrum of **13d** (400 MHz, $CDCl_3$)

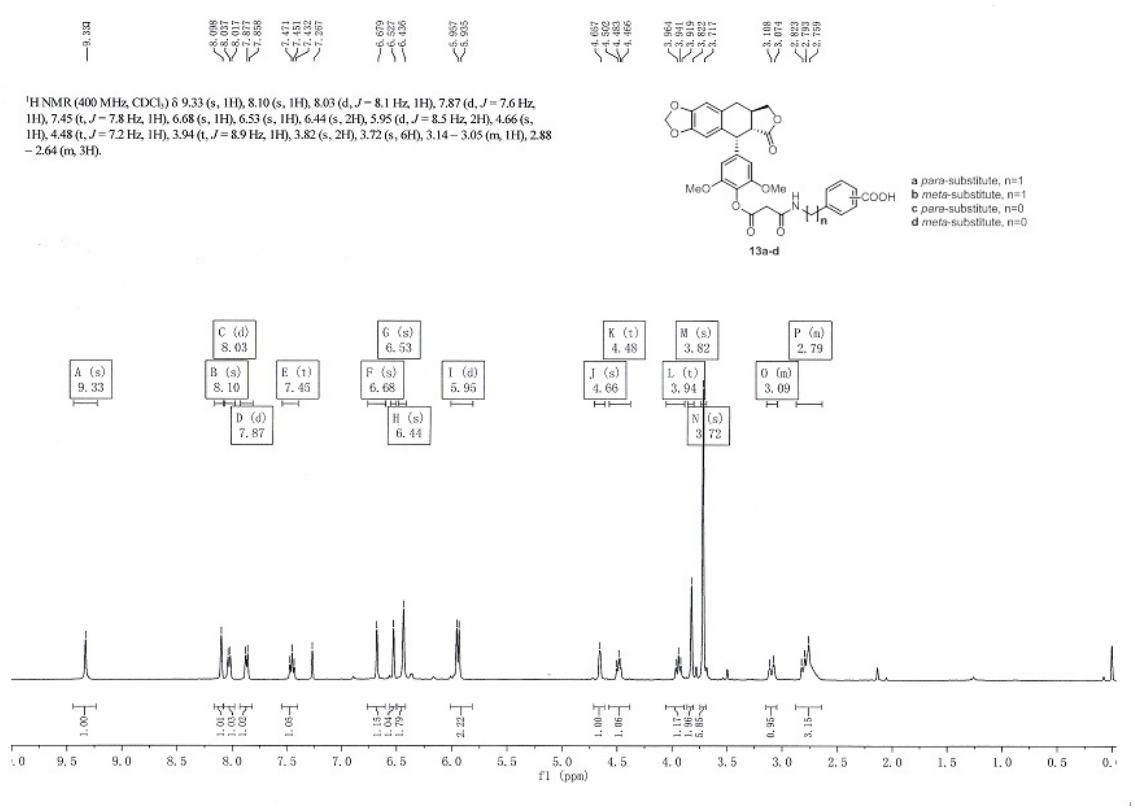


Figure S22. ^{1}H NMR Spectrum of **14a** (400 MHz, $CDCl_3$)

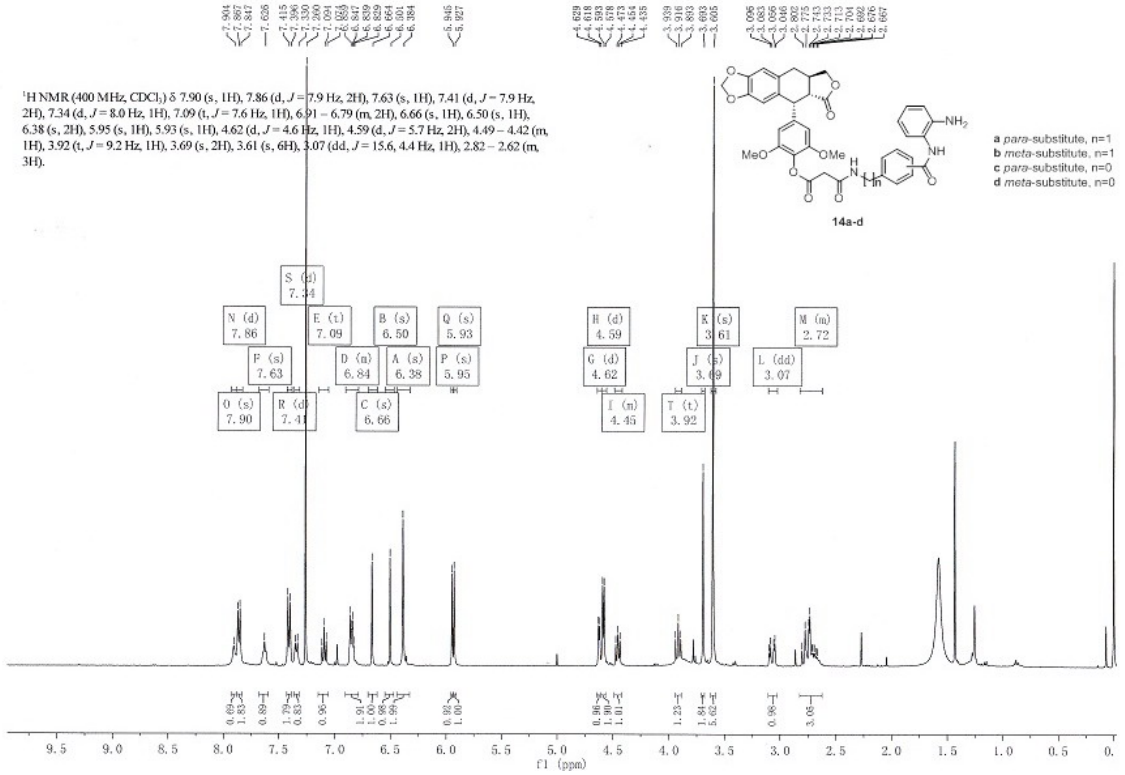


Figure S23. ^{1}H NMR Spectrum of **14b** (400 MHz, $CDCl_3$)

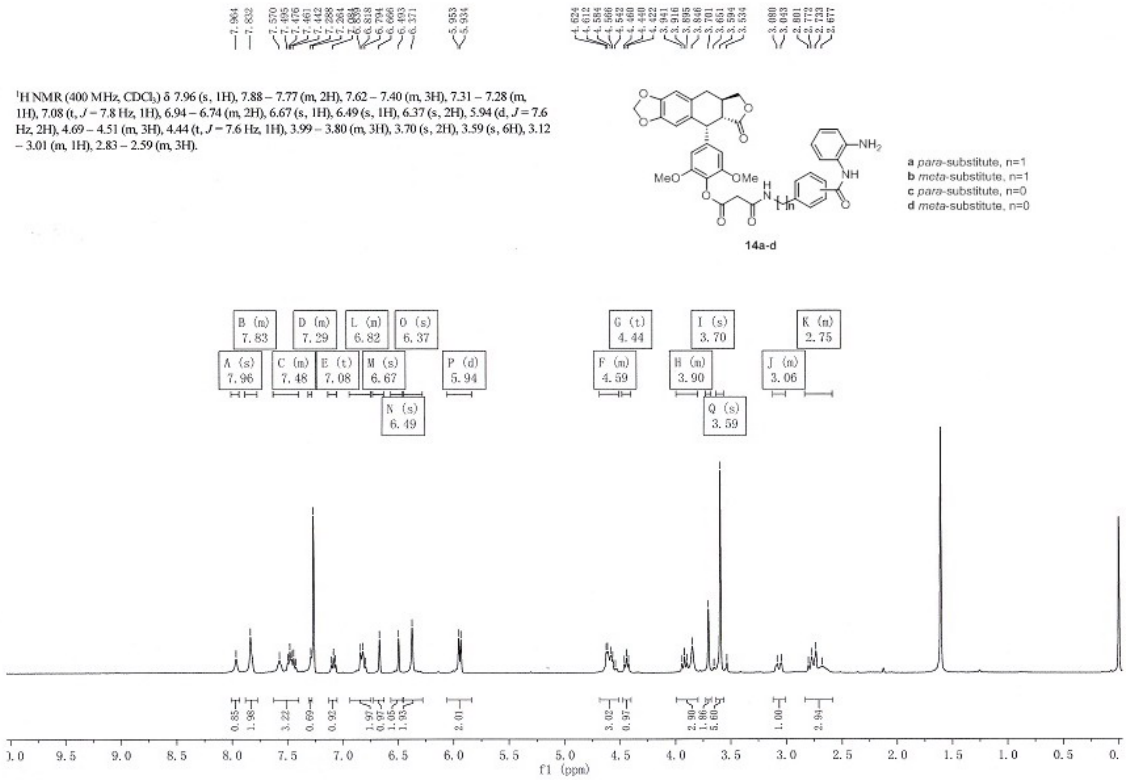


Figure S24. ^{1}H NMR Spectrum of **14c** (400 MHz, $CDCl_3$)

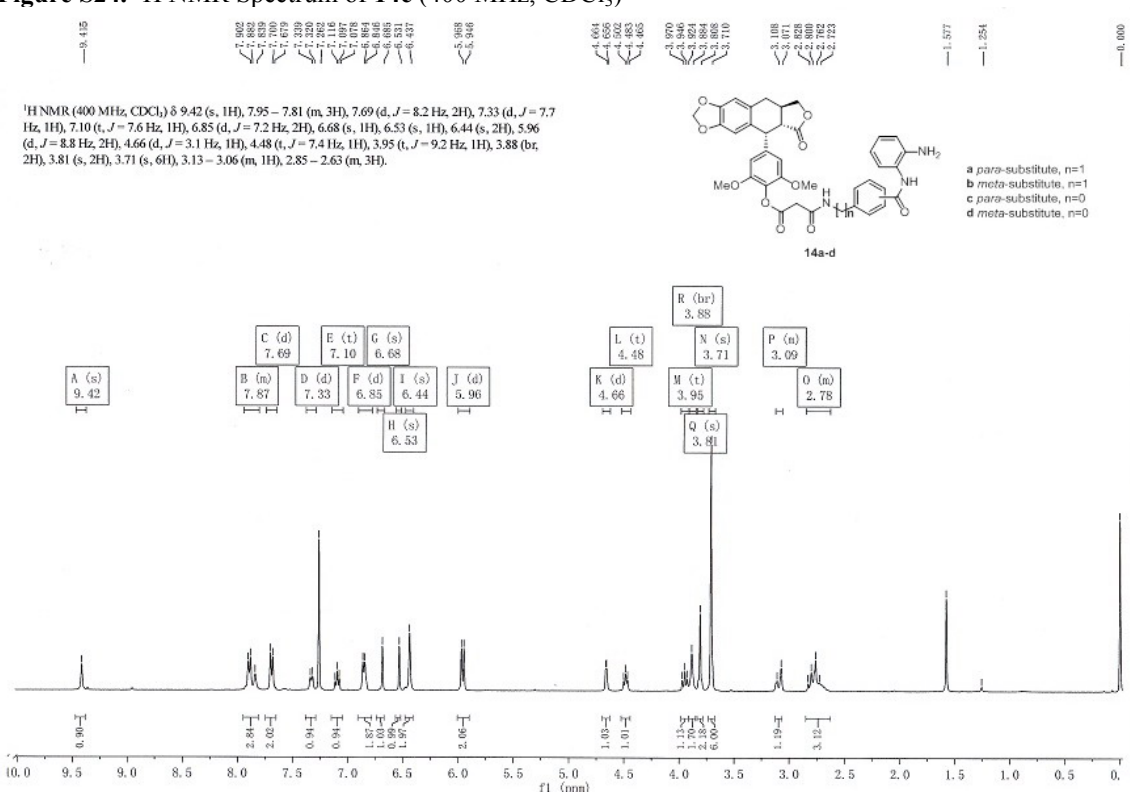


Figure S25. ^{1}H NMR Spectrum of **14d** (400 MHz, $CDCl_3$)