



HOKKAIDO UNIVERSITY

Title	Synthesis and the Intestinal Glucosidase Inhibitory Activity of 2-Aminoresorcinol Derivatives toward an Investigation of Its Binding Site
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General methods.

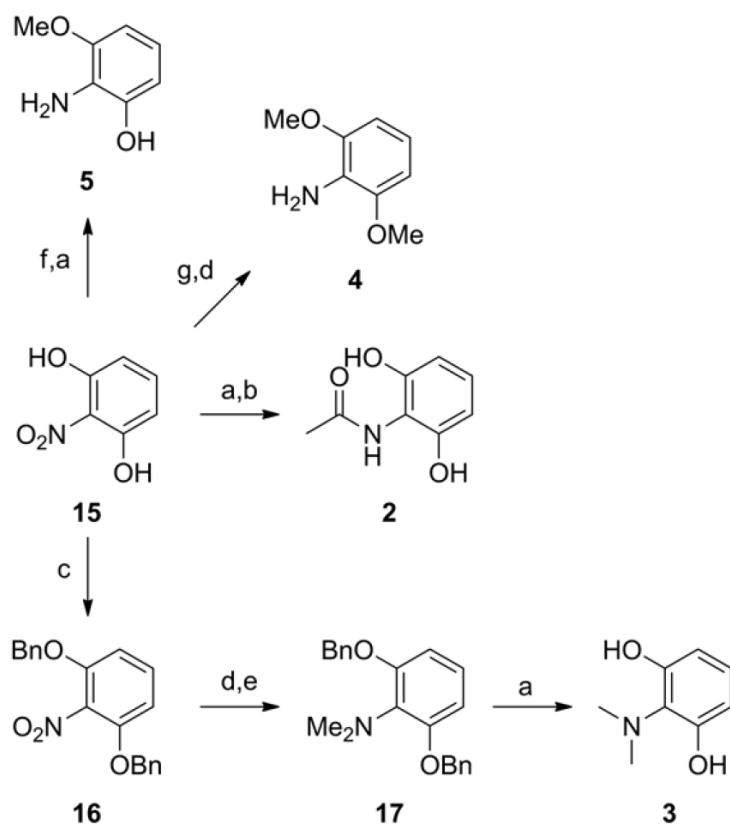
Intestinal acetone powder from rat was purchased from Sigma-Aldrich Co. All other commercially available chemicals were purchased from Wako Pure Chem. Ind. Ltd. and used without further purification. Structures of the synthetic compounds were determined by NMR and Mass spectrometry. Bruker AMX500 or Jeol JNM-EX 270 was used to obtain NMR spectrum and either tetramethylsilane (TMS) or residual solvent peak was used as an internal standard (^1H NMR: TMS 0.00 ppm for CDCl_3 , acetone- d_6 2.04 ppm, CD_3OD 3.30 ppm, DMSO- d_6 2.50 ppm; ^{13}C NMR: CDCl_3 77.0 ppm, acetone- d_6 29.8 ppm, CD_3OD 49.0 ppm, DMSO- d_6 39.5 ppm). Jeol JMS SX-102A (FAB-MS) or Jeol JMS-T100GCV (FD-MS) or Thermo Scientific Exactive (ESI-MS) was used to obtain mass spectrum. Absorbance was measured by SynergyTM MX (Bio-tech Instruments Inc.,) microplate reader.

Intestinal α -glucosidase inhibitory activity determination.

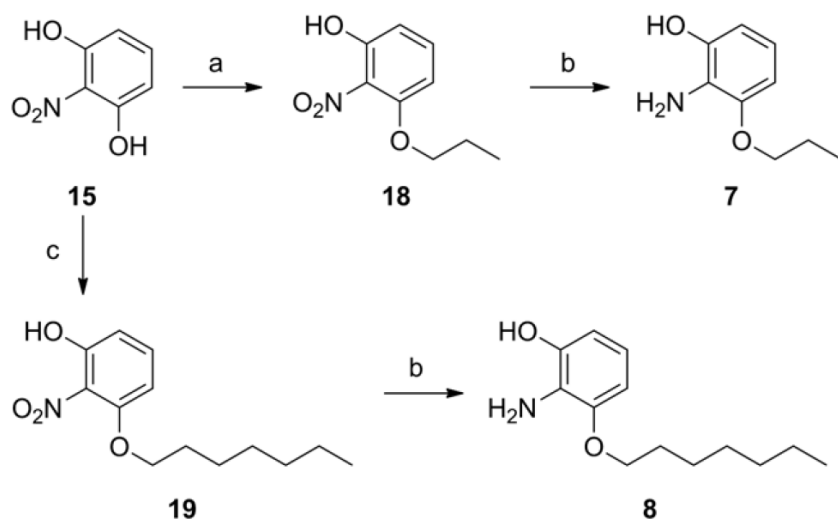
Sucrase inhibition (SI) and maltase inhibition (MI) designates an inhibition of sucrose and maltose hydrolyzing activity in rat intestinal glucosidase complexes. Rat intestinal acetone powder (10 g) was homogenized in 70 mL of 0.1 M phosphate buffer (pH 7.0, 5 mM EDTA), centrifuged (15,000 g $\times$ 30 min) and the supernatant was used as the crude intestinal α -glucosidase. The reaction mixture consisting from crude enzyme, substrate (56 mM sucrose or 3.5 mM maltose in phosphate buffer) and the test sample (in 50% DMSO aq.) was mixed and incubated at 37 °C as written in bellow table. The reaction was stopped by adding 0.75 mL of 2 M Tris-HCl buffer (pH 7.0) and then passed through a short column of Cosmosil 75C₁₈-OPN (Nacalai Tesque, Inc.). The amount of liberated glucose was measured by glucose oxidase method using Glucose CII-test Wako (Wako Pure Chem. Co.).

	Crude enzyme	Substrate	Sample	Incubation time
SI assay	0.20 mL	0.20 mL	0.1 mL	30 min
MI assay	0.05 mL	0.35 mL	0.1 mL	15 min

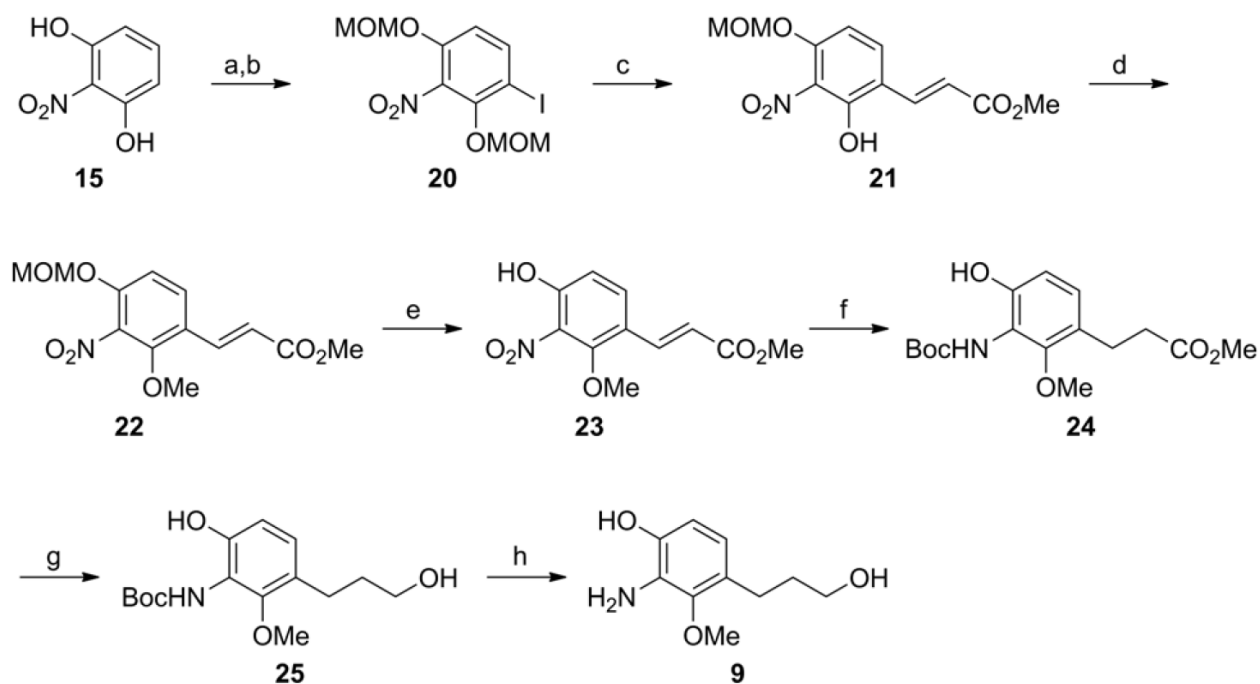
Synthesis of 2-aminoresorcinol derivatives.



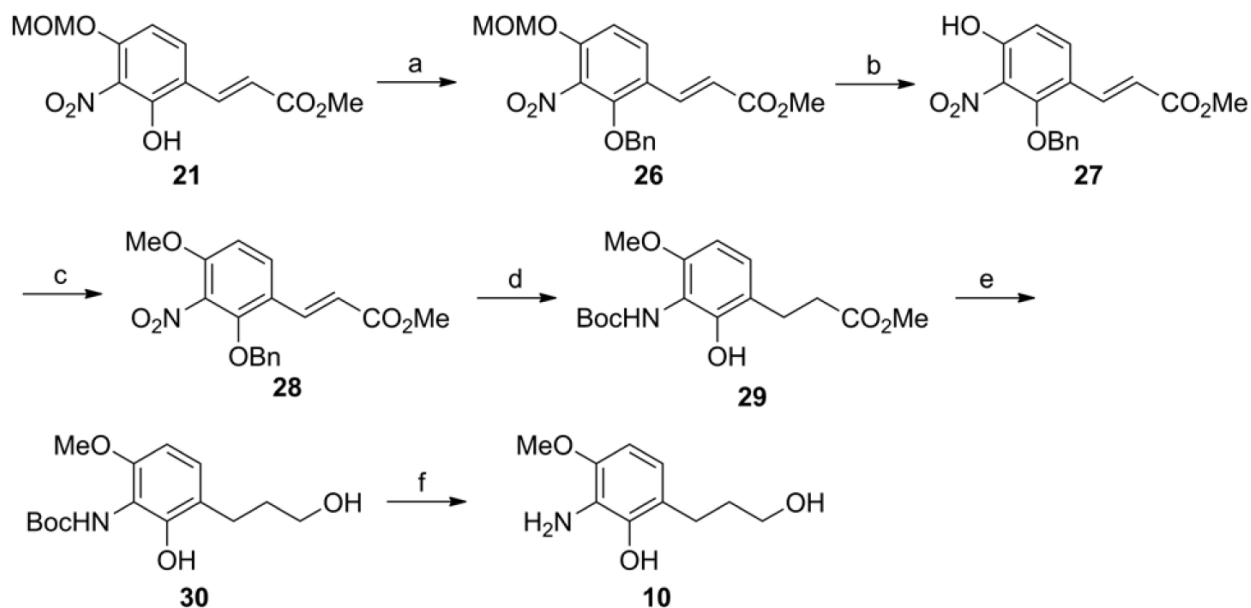
Scheme S1. Reagent and conditions: (a) H_2 , Pd/C (10%), MeOH, rt (b) AcCl, MeOH, -40°C to rt (c) BnBr, K_2CO_3 , MeCN, rt (d) Zn, AcOH, rt (e) MeI, K_2CO_3 , acetone, reflux (f) MeOH, DIAD, PPh_3 , THF, rt (g) Me_2SO_4 , K_2CO_3 , acetone, reflux.



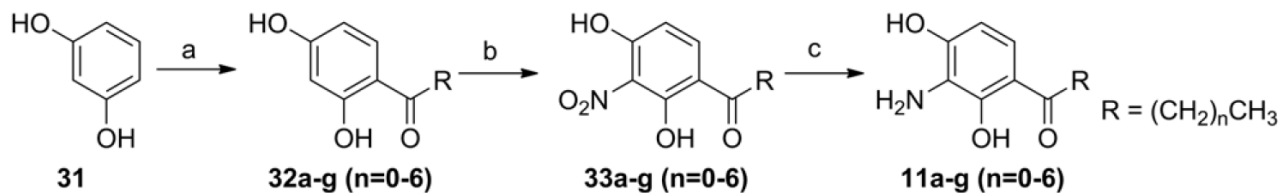
Scheme S2. Reagent and conditions: (a) propanol, DIAD, PPh₃, THF, rt (b) H₂, 10% Pd/C, MeOH, rt (c) heptanol, DIAD, PPh₃, THF, rt.



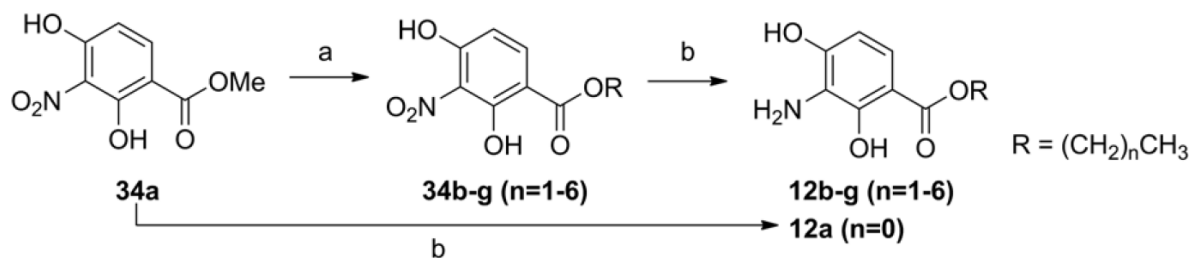
Scheme S3. Reagent and conditions: (a) *N*-iodosuccinimide, trifluoroacetic acid, rt (b) MOMCl, *i*-Pr₂NEt, DMF, rt (c) methyl acrylate, Et₃N, Pd(OAc)₂, tri-*O*-tolylphosphine, MeCN, 70°C (d) MeI, K₂CO₃, acetone, reflux (e) AcCl, MeOH, rt (f) H₂, Pd/C, Boc₂O, THF, rt (g) LiAlH₄, THF, rt (h) H₂O, reflux.



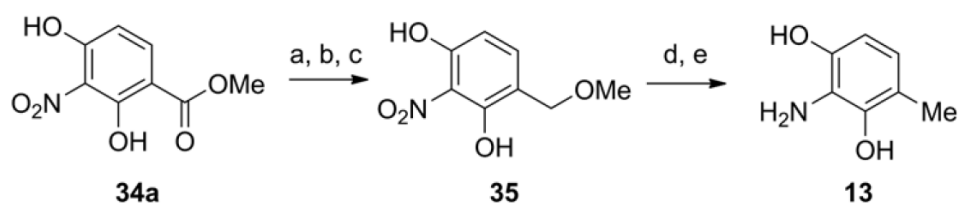
Scheme S4. Reagent and conditions: (a) BnBr, K₂CO₃, MeCN, reflux (b) AcCl, MeOH, rt (c) MeI, K₂CO₃, acetone, reflux (d) H₂, Pd/C, Boc₂O, THF, rt (e) LiAlH₄, THF, rt (f) H₂O, reflux.



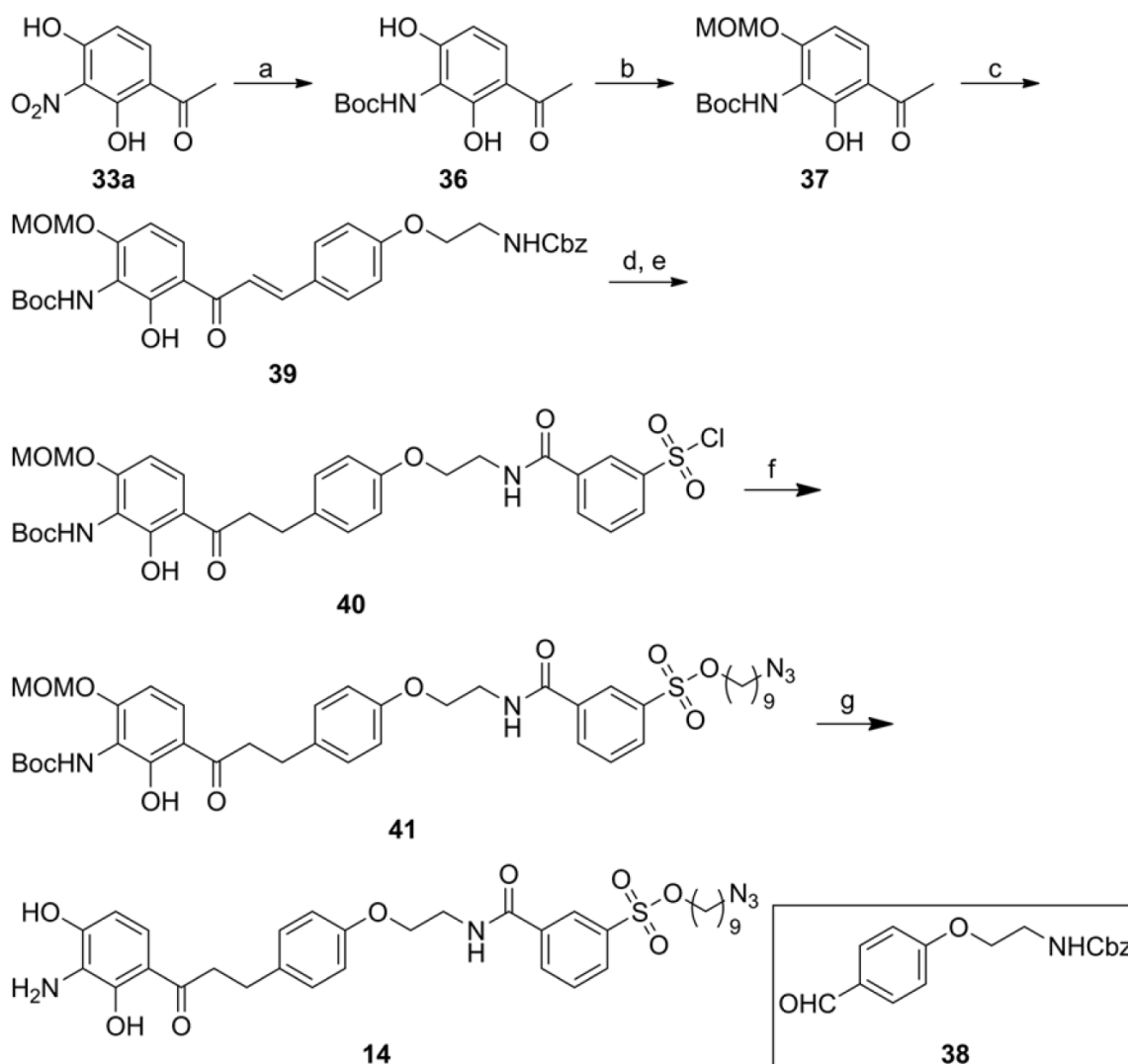
Scheme S5. Reagent and conditions: (a) BF₃·Et₂O, RCO₂H, 80°C (b) 70% HNO₃ aq., AcOH, rt (c) H₂, Pd/C, MeOH, rt.



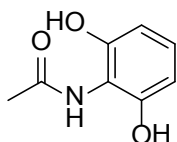
Scheme S6. Reagent and conditions: (a) ROH, H₂SO₄, reflux (b) H₂, Pd/C, MeOH, rt.



Scheme S7. Reagent and conditions: (a) MOMCl, *i*-Pr₂NEt, THF, rt (b) LiAlH₄, THF, Et₂O, 0°C (c) conc. HCl, MeOH, 60°C (d) 10% Pd/C, H₂, MeOH, rt (e) HCl, 1,4-dioxane, rt.

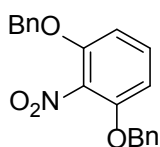


Scheme S8. Reagent and conditions: (a) H₂, Pd/C, Boc₂O, THF, rt (b) MOMCl, *i*-Pr₂NEt, DMF, 0°C (c) **36**, 50% NaOH aq., 1,4-dioxane, rt (d) H₂, Pd/C, MeOH, rt (e) 3-(chlorosulfonyl)benzoyl chloride, pyridine, CH₂Cl₂, 0 °C (f) 9-azidononanol, DMAP, CH₂Cl₂, 0°C (g) HCl, 1,4-dioxane, 0°C.

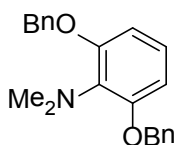


N-(2,6-Dihydroxyphenyl)acetamide (**2**).¹⁹ 2-Nitroresorcinol (**15**, 1.0 g, 6.4 mmol) was dissolved in MeOH (20 mL) and 10% Pd/C (100 mg) was added to the solution. After stirring overnight under hydrogen atmosphere, the reaction mixture was passed through Celite[®] pad. The filtrate was cooled to -40°C and AcCl (2 mL) was added dropwise. The mixture was warmed to rt and stirred for 5 hours. The reaction mixture was evaporated and suspended in acetone. The resulting suspension was filtered and the filtrate was dried to give **2** (0.57 g, 53%).

FD-MS (positive): m/z (%) 167 (100, $[M]^+$). ¹H-NMR (270 MHz, acetone- d_6 , rt): 2.25 (s, 3H, CH₃), 6.38 (d, 2H, J = 8.0 Hz, H-3,5), 6.84 (t, 1H, J = 8.0 Hz, H-4) ppm.



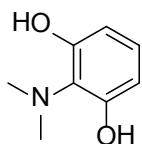
1,3-Bis(benzyloxy)-2-nitrobenzene (**16**). 2-Nitroresorcinol (**15**, 1.66 g, 10.7 mmol) was dissolved in MeCN (40 mL) and K₂CO₃ (5.0 g, 36.2 mmol), benzyl bromide (3.0 mL, 25.2 mmol) was added. After stirring for 10 days, the reaction mixture was filtered and the filtrate was evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to give **16** (2.67 g, 74%). HR-FD-MS (positive): Found m/z 335.1151 ($[M]^+$, calcd. for C₂₀H₁₇NO₄ 335.1158); ¹H-NMR (270 MHz, CDCl₃, rt): 5.16 (s, 4H, OCH₂), 6.64 (d, 2H, J = 8.5 Hz, H-3,5), 7.23 (t, 1H, J = 8.5 Hz, H-4), 7.31-7.38 (m, 10H, Ph) ppm.



2,6-Bis(benzyloxy)-*N,N*-dimethylaniline (**17**). Compound **16** (934.5 mg, 2.8 mmol) was dissolved in

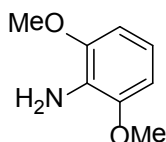
acetic acid (50 mL) and zinc powder (10.8 g, 165.1 mmol) was added. After stirring for 30 min, the reaction mixture was passed through Celite[®] pad and evaporated. The residue was dissolved in acetone (5 mL) and K₂CO₃ (336 mg, 2.4 mmol), iodomethane (0.39 mL, 5.93 mmol) was added. The mixture was refluxed overnight, then cooled to rt, filtered and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to give **17** (730.4 mg, 79%).

HR-FD-MS (positive): Found m/z 333.1727 ($[M]^+$, calcd. for C₂₂H₂₃NO₂ 333.1729); ¹H-NMR (270 MHz, CDCl₃, rt): 2.89 (s, 6H, NMe₂), 5.14 (s, 4H, OCH₂), 6.56 (d, 2H, J = 8.3 Hz, H-3,5), 6.91 (t, 1H, J = 8.3 Hz, H-4), 7.30-7.46 (m, 10H, Ph) ppm; ¹³C-NMR (67.5 MHz, CDCl₃, rt): 43.6, 70.7, 107.11, 107.13, 124.6, 127.1, 127.6, 128.4, 137.6, 156.8 ppm.



2,6-Dihydroxy-N,N-dimethylaniline (3). Compound **17** (730 mg, 2.2 mmol) was dissolved in MeOH (15 mL) and 10% Pd/C (39.3 mg) was added. After stirring overnight under hydrogen atmosphere, the resulting mixture was passed through Celite[®] pad and evaporated. The residue was purified by silica-gel column chromatography (CHCl₃/MeOH) to give **3** (49.8 mg, 15%).

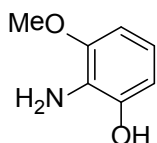
HR-FD-MS (positive): Found m/z 153.0771 ($[M]^+$, calcd. for C₈H₁₁NO₂ 153.0790); ¹H-NMR (270 MHz, CD₃OD, rt): 2.71 (s, 6H, NMe₂), 6.22 (d, 2H, J = 8.1 Hz, H-3,5), 6.74 (t, 1H, J = 8.1 Hz, H-4) ppm; ¹³C-NMR (67.5 MHz, CD₃OD, rt): 43.6, 107.1, 126.9, 127.8, 156.7 ppm.



2,6-Dimethoxyaniline (4).²⁰⁾ 2-Nitroresorcinol (**15**, 833 mg, 5.37 mmol) was dissolved in acetone (15 mL) and K₂CO₃ (1.5 g, 10.87 mmol), dimethyl sulfate (1.05 mL, 12 mmol) was added and refluxed overnight. The reaction mixture was filtered and evaporated. The residue was partially purified by

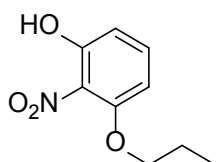
silica-gel column chromatography (Hexane/EtOAc) and the crude product (1.11 g) was dissolved in acetone (40 mL). Zinc powder (9.6 g) was added to the solution and the mixture was stirred for 30 min. The reaction mixture was passed through Celite[®] pad and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to give **4** (240 mg, 29%).

HR-FD-MS (positive): Found m/z 153.0771 ($[M]^+$, calcd. for $C_8H_{11}NO_2$ 153.0790); 1H -NMR (270 MHz, $CDCl_3$, rt): 3.85 (6H, s), 6.53 (2H, d, $J = 8.1$ Hz), 6.69 (1H, t, $J = 8.1$ Hz) ppm



*2-Amino-3-methoxyphenol (5).*²¹⁾ 2-Nitroresorcinol (**15**, 250 mg, 1.60 mmol) was dissolved in THF (15 mL) and MeOH (60 μ L, 1.90 mmol), Ph_3P (420 mg, 1.60 mmol) was added. After stirring for 10 min under nitrogen atmosphere, diisopropyl azodicarboxylate (DIAD, 0.32 mL, 1.60 mmol) was added and stirred overnight under nitrogen atmosphere. The reaction mixture was evaporated and the residue was partially purified by silica-gel column chromatography (Hexane/EtOAc). The crude product (119.3 mg) was dissolved in MeOH (5 mL), 10% Pd/C (12.7 mg) was added and stirred overnight under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and the filtrate was evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to give **5** (43.4 mg, 20%).

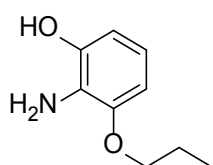
HR-FD-MS (positive): Found m/z 139.0630 ($[M]^+$, calcd. for $C_7H_9NO_2$ 139.0633); 1H -NMR (270 MHz, $CDCl_3$, rt): 3.84 (3H, s), 4.28 (2H, s), 6.46 (2H, d, $J = 8.0$ Hz), 6.65 (1H, t, $J = 8.0$ Hz) ppm



2-Nitro-3-propoxyphenol (18). To the solution of 2-nitroresorcinol (**15**, 0.25 g, 1.6 mmol) dissolved in THF (15 mL), propanol (0.12 mL, 1.6 mmol) and Ph_3P (0.42 g, 1.6 mmol) were added and stirred

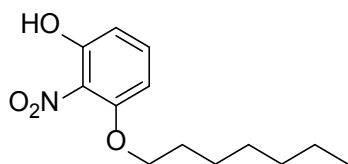
for 10 min under argon atmosphere. To this mixture, DIAD (0.32 mL, 1.6 mmol) was added and further stirred overnight under argon atmosphere. The reaction mixture was evaporated and purified by silica-gel column chromatography (Hexane/EtOAc) to give **18** (136.9 mg, 43%).

HR-FD-MS (positive): Found m/z 197.0698 ($[M]^+$, calcd. for $C_9H_{11}NO_4$ 197.0688); 1H -NMR (270 MHz, $CDCl_3$, rt): 1.09 (3H, t, $J = 7.4$ Hz), 1.94-1.81 (2H, m), 4.03 (2H, t, $J = 6.3$ Hz), 6.52 (1H, d, $J = 8.4$ Hz), 6.68 (1H, d, $J = 8.4$ Hz), 7.37 (1H, t, $J = 8.4$ Hz), 10.19 (1H, s), ppm; ^{13}C -NMR (67.5 MHz, $CDCl_3$, rt): 10.3, 22.2, 71.2, 104.4, 110.1, 135.4, 155.2, 155.3 ppm



2-Amino-3-propoxyphenol (7). Compound **18** (128 mg, 0.65 mmol) was dissolved in MeOH (5 mL), 10% Pd/C (11.7 mg) was added and stirred overnight under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to give **7** (60.7 mg, 56%).

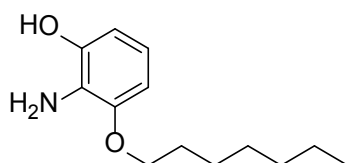
HR-FD-MS (positive): Found m/z 167.0943 ($[M]^+$, calcd. for $C_9H_{13}NO_2$ 167.0946); 1H -NMR (270 MHz, CD_3OD , rt): 1.01 (3H, t, $J = 7.4$ Hz), 1.88-1.75 (2H, m), 4.00 (2H, t, $J = 6.6$ Hz), 6.55 (1H, d, $J = 8.4$ Hz), 6.56 (1H, d, $J = 8.4$ Hz), 7.09 (1H, t, $J = 8.4$ Hz) ppm; ^{13}C -NMR (67.5 MHz, CD_3OD , rt): 10.7, 23.3, 71.7, 104.4, 109.2, 109.3, 129.8, 152.5, 154.0 ppm



3-Heptyloxy-2-nitrophenol (19). To the solution of 2-nitroresorcinol (**15**, 0.25 g, 1.6 mmol) dissolved in THF (15 mL), heptanol (0.23 mL, 1.6 mmol) and Ph_3P (0.42 g, 1.6 mmol) were added and stirred for 10 min under argon atmosphere. To this mixture, DIAD (0.32 mL, 1.6 mmol) was added and further stirred overnight under argon atmosphere. The reaction mixture was evaporated and purified

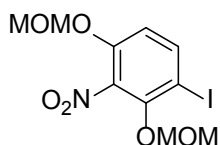
by silica-gel column chromatography (Hexane/EtOAc) to give **19** (203.6 mg, 50%).

HR-FD-MS (positive): Found m/z 253.1306 ($[M]^+$, calcd. for $C_{13}H_{19}NO_4$ 253.1314); 1H -NMR (270 MHz, $CDCl_3$, rt): 0.90 (3H, t, $J = 6.5$ Hz), 1.56-1.26 (8H, m), 1.85 (2H, m), 4.06 (2H, t, $J = 6.4$ Hz), 6.52 (1H, d, $J = 8.5$ Hz), 6.67 (1H, d, $J = 8.5$ Hz), 7.37 (1H, t, $J = 8.5$ Hz), 10.19 (1H, s) ppm; ^{13}C -NMR (67.5 MHz, $CDCl_3$, rt): 14.0, 22.5, 25.8, 28.9, 29.0, 31.7, 69.9, 104.4, 105.7, 110.2, 135.5, 155.4, 155.5 ppm



2-Amino-3-heptyloxyphenol (8). Compound **19** (198.2 mg, 0.78 mmol) was dissolved in MeOH (5 mL), 10% Pd/C (14 mg) was added and stirred overnight under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated to give **8** (193.8 mg, quant.)

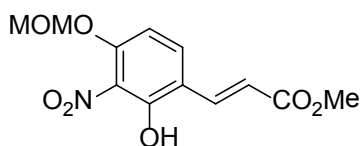
HR-FD-MS (positive): Found m/z 223.1544 ($[M]^+$, calcd. for $C_{13}H_{21}NO_2$ 223.1572); 1H -NMR (270 MHz, $CDCl_3$, rt): 0.89 (3H, t, $J = 6.7$ Hz), 1.55-1.22 (8H, m), 1.80 (2H, m), 3.97 (2H, t, $J = 6.5$ Hz), 4.06 (2H, br s), 6.45 (2H, d, $J = 8.0$ Hz), 6.65 (1H, t, $J = 8.0$ Hz) ppm; ^{13}C -NMR (67.5 MHz, $CDCl_3$, rt): 14.0, 22.5, 25.9, 29.0, 29.3, 31.7, 68.5, 104.0, 108.9, 119.8, 121.5, 145.9, 148.8 ppm.



1-Iodo-2,4-bis(methoxymethoxy)-3-nitrobenzene (20).²²⁾ 2-Nitroresorcinol (**15**, 1.61g, 10.4 mmol) was dissolved in TFA (45 mL) and NIS (2.28 g, 10.1 mmol) was added in portion at 0°C. After stirring for 30 min at rt, cold water was added to the reaction mixture and extracted with toluene. The organic layer was washed with sat. $NaHSO_3$ aq., dried over sodium sulfate and evaporated to dryness. The residue was dissolved in DMF (45 mL), i -Pr₂NEt (4.5 mL, 27.6 mmol) and MOMCl (2.55 mL, 31.6 mmol) was added and stirred for 5 hours at rt. Water was added to the reaction mixture and the

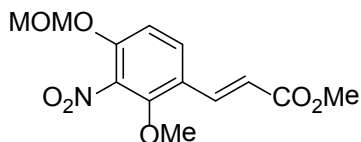
resulting solution was extracted with EtOAc. Organic layer was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc = 5/1) to obtain **20** (3.56g, 93%).

FD-MS (positive): m/z 369 ($[M]^+$); $^1\text{H-NMR}$ (270 MHz, CDCl_3 , rt): 3.48 (3H, s), 3.54 (3H, s), 5.14 (2H, s), 5.22 (2H, s), 6.89 (1H, d, $J = 9.0$ Hz), 7.79 (1H, d, $J = 9.0$ Hz) ppm.



Methyl 2-hydroxy-4-methoxymethoxy-3-nitrocinnamate (21). Compound **20** (1.97 g, 5.3 mmol) was dissolved in MeCN (70 mL) and Methyl acrylate (2.86 mL, 30 mmol), tri-*O*-tolylphosphine (1.2 g, 5.2 mmol), TEA (7.0 mL, 52 mmol), $\text{Pd}(\text{OAc})_2$ (590 mg, 2.6 mmol) was added. The mixture was stirred for 6 hours at 70°C under nitrogen atmosphere. Sat. NH_4Cl aq. was added and the reaction mixture was extracted with EtOAc. Organic layer was washed with brine, dried over sodium sulfate and evaporated to dryness. The residue was partly purified by silica-gel column chromatography (Hexane/EtOAc = 4/1) to obtain **21** (1.54 g, quant).

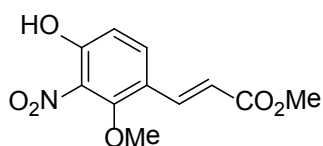
HR-FD-MS (positive): Found m/z 283.0684 ($[M]^+$, calcd. for $\text{C}_{12}\text{H}_{13}\text{NO}_7$ 283.0692); $^1\text{H-NMR}$ (270 MHz, CDCl_3 , rt): 3.54 (3H, s), 3.81 (3H, s), 5.33 (2H, s), 6.52 (1H, d, $J = 16.2$ Hz), 6.84 (1H, d, $J = 9.0$ Hz), 7.63 (1H, d, $J = 9.0$ Hz), 7.91 (1H, d, $J = 16.2$ Hz) ppm



Methyl 2-methoxy-4-methoxymethoxy-3-nitrocinnamate (22). Compound **21** (1.45 g, 5.12 mmol) was dissolved in acetone (5 mL) and K_2CO_3 (307 mg, 2.2 mmol), MeI (0.36 mL, 5.8 mmol) was added. The mixture was refluxed for 5 hours, cooled to rt, filtered and the filtrate was evaporated to dryness. Water was added to the residue and the resulting suspension was extracted by EtOAc. The organic

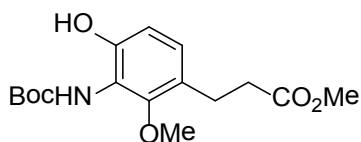
layer was washed with brine, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc = 5/1) to obtain **22** (0.87 g, 58%).

HR-FD-MS (positive): Found m/z 297.0837 ($[M]^+$, calcd. for $C_{13}H_{15}NO_7$ 297.0849); 1H -NMR (270 MHz, $CDCl_3$, rt): 3.49 (3H, s), 3.82 (3H, s), 3.90 (3H, s), 5.26 (2H, s), 6.46 (1H, d, $J = 16.2$ Hz), 7.07 (1H, d, $J = 9.1$ Hz), 7.58 (1H, d, $J = 9.1$ Hz), 7.79 (1H, d, $J = 16.2$ Hz) ppm.



Methyl 4-hydroxy-2-methoxy-3-nitrocinnamate (23). Compound **22** (1.61 g, 5.43 mmol) was dissolved in MeOH (45 mL) and AcCl (3.5 mL) was added to the solution. The mixture was stirred for 8.5 hours at rt and then evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc = 3/1) to obtain **23** (1.31 g, 95%).

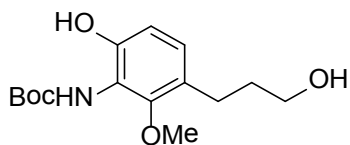
HR-FD-MS (positive): Found m/z 253.0592 ($[M]^+$, calcd. for $C_{11}H_{11}NO_6$ 253.0586); 1H -NMR (270 MHz, acetone- d_6 , rt): 3.71 (3H, s), 3.85 (3H, s), 6.48 (1H, d, $J = 16.1$ Hz), 6.94 (1H, d, $J = 8.9$ Hz), 7.71 (1H, d, $J = 16.1$ Hz), 7.77 (1H, d, $J = 8.9$ Hz) ppm.



Methyl 3-(3-((tert-butoxycarbonyl)amino)-4-hydroxy-2-methoxyphenyl)propanoate (24). Compound **23** (1.25 g, 4.9 mmol) was dissolved in THF (35 mL) and 10% Pd on activated carbon (0.18 g), Boc_2O (2.7 g, 12.4 mmol) were added. The mixture was stirred overnight under hydrogen atmosphere. MeOH was added to the reaction mixture and the resulting suspension was passed through Celite[®] pad. The residue was partially purified by silica-gel column chromatography (Hexane/EtOAc = 2/1) to obtain **24** (1.21 g, 75%).

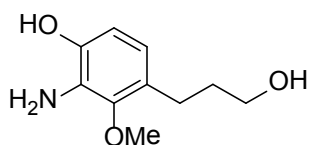
HR-FD-MS (positive): Found m/z 325.1504 ($[M]^+$, calcd. for $C_{16}H_{23}NO_6$ 325.1525); 1H -NMR (270

MHz, CDCl₃, rt): 1.54 (9H, s), 2.58 (2H, t, $J = 7.8$ Hz), 2.88 (2H, t, $J = 7.8$ Hz), 3.67 (3H, s), 3.75 (3H, s), 6.72 (1H, d, $J = 8.5$ Hz), 6.86 (1H, d, $J = 8.5$ Hz), 6.97 (1H, br s), 9.13 (1H, br s) ppm.



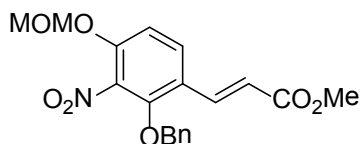
3-(N-(tert-Butoxycarbonyl)-3-amino-4-hydroxy-2-methoxyphenyl)propanol (25). Compound **24** (1.17 g, 3.6 mmol) was dissolved in THF (30 mL) and LiAlH₄ (0.15 g, 4.0 mmol) was added at 0°C. The mixture was stirred overnight and EtOAc was added to the reaction mixture. The resulting solution was washed with 1 M HCl aq. and brine, then dried over sodium sulfate, and evaporated to dryness. The residue was purified by silica-gel column chromatography (CHCl₃/MeOH = 100/1) to obtain **25** (0.86 g, 80%).

HR-FD-MS (positive): Found m/z 297.1548 ($[M]^+$, calcd. for C₁₅H₂₃NO₅ 297.1576); ¹H-NMR (270 MHz, CDCl₃, rt): 1.54 (9H, s), 1.87-1.77 (2H, m), 2.66 (2H, t, $J = 7.4$ Hz), 3.58 (2H, t, $J = 6.2$ Hz), 3.75 (3H, s), 6.75 (1H, d, $J = 8.5$ Hz), 6.88 (1H, d, $J = 8.5$ Hz), 6.96 (1H, br s), 9.12 (1H, br s) ppm.



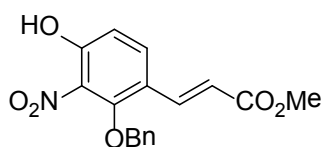
3-(3-Amino-4-hydroxy-2-methoxyphenyl)propanol (9). Compound **25** (48 mg, 0.16 mmol) was suspended in water (3.2 mL) and refluxed for two hours. The reaction mixture was cooled and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and evaporated to obtain pure **9** (17.4 mg, 55%).

HR-FD-MS (positive): Found m/z 197.1042 ($[M]^+$, calcd. for C₁₀H₁₅NO₃ 197.1052); ¹H-NMR (270 MHz, CD₃OD, rt): 1.78-1.67 (2H, m), 2.53 (2H, t, $J = 7.7$ Hz), 3.51 (2H, t, $J = 6.6$ Hz), 3.69 (3H, s), 6.36 (1H, d, $J = 8.2$ Hz), 6.41 (1H, d, $J = 8.2$ Hz) ppm.



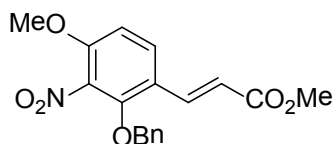
Methyl 2-benzyloxy-4-methoxymethoxy-3-nitrocinnamate (26). Compound **21** was dissolved in MeCN (30 mL) and K₂CO₃ (1.12 g, 8.11 mmol), BnBr (0.90 mL, 7.57 mmol) was added. The mixture was refluxed for 90 min, cooled to rt and filtered. The filtrate was evaporated to dryness and the residue was purified by silica-gel column chromatography (Hexane/EtOAc = 4/1) to obtain **26** (2.10 g, 87%).

HR-FD-MS (positive): Found m/z 373.1156 ($[M]^+$, calcd. for C₁₉H₁₉NO₇ 373.1162); ¹H-NMR (270 MHz, CDCl₃, rt): 3.50 (3H, s), 3.80 (3H, s), 4.99 (2H, s), 5.27 (2H, s), 6.42 (1H, d, J = 16.2 Hz), 7.10 (1H, d, J = 8.9 Hz), 7.43-7.36 (5H, m), 7.59 (1H, d, J = 8.9 Hz), 7.82 (1H, d, J = 16.2 Hz) ppm.



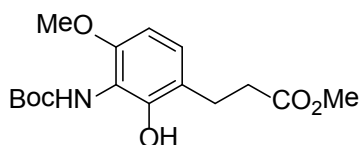
Methyl 2-benzyloxy-4-hydroxy-3-nitrocinnamate (27). Compound **26** (2.09 g, 5.6 mmol) was dissolved in MeOH (50 mL) and AcCl (4.4 mL) was added. After stirring overnight, water was added and the reaction mixture was extracted with EtOAc. Organic layer was washed with brine, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc = 2/1) to obtain **27** (1.51 g, 82%).

HR-FD-MS (positive): Found m/z 329.0879 ($[M]^+$, calcd. for C₁₇H₁₅NO₆ 329.0899); ¹H-NMR (270 MHz, CDCl₃, rt): 3.80 (3H, s), 5.00 (2H, s), 6.36 (1H, d, J = 16.2 Hz), 6.98 (1H, d, J = 9.0 Hz), 7.51-7.38 (5H, m), 7.71 (1H, d, J = 9.0 Hz), 7.90 (1H, d, J = 16.2 Hz), 10.02 (1H, s) ppm.



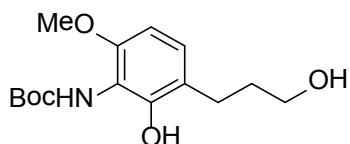
Methyl 2-benzyloxy-4-methoxy-3-nitrocinnamate (28). Compound **27** (1.51 g, 4.59 mmol) was dissolved in acetone (20 mL) and K_2CO_3 (275 mg, 1.99 mmol), MeI (0.32 mL, 5.1 mmol) was added. The mixture was refluxed for 5 hours and K_2CO_3 (100 mg, 0.72 mmol), MeI (0.20 mL, 3.2 mmol) was added again. After refluxing overnight, the reaction mixture was cooled and filtered. The filtrate was evaporated, water was added to the residue and the suspension was extracted with EtOAc. Organic layer was washed with brine, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc = 3/1) to obtain **28** (1.80 g, quant).

HR-FD-MS (positive): Found m/z 343.1045 ($[M]^+$, calcd. for $C_{18}H_{17}NO_6$ 343.1056); 1H -NMR (270 MHz, $CDCl_3$, rt): 3.54 (3H, s), 3.81 (3H, s), 5.33 (2H, s), 6.52 (1H, d, $J = 16.2$ Hz), 6.84 (1H, d, $J = 9.0$ Hz), 7.63 (1H, d, $J = 9.0$ Hz), 7.91 (1H, d, $J = 16.2$ Hz) ppm.



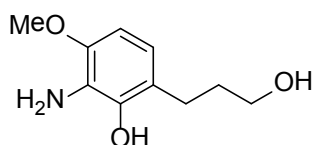
Methyl 3-((tert-butoxycarbonyl)amino)-2-hydroxy-4-methoxyphenyl)propanoate (29). To the solution of **28** (1.80 g, 5.24 mmol) dissolved in THF (40 mL), 10% Pd on carbon (185 mg), Boc_2O (1.9 g, 8.71 mmol) was added and stirred overnight under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad, evaporated and the residue was purified by silica-gel column chromatography (Hexane/EtOAc = 4/1) to obtain **29** (1.29 g, 76%).

HR-FD-MS (positive): Found m/z 325.1521 ($[M]^+$, calcd. for $C_{16}H_{23}NO_6$ 325.1525); 1H -NMR (270 MHz, $CDCl_3$, rt): 1.53 (9H, s), 2.65 (2H, t, $J = 7.6$ Hz), 2.93 (2H, t, $J = 7.6$ Hz), 3.66 (3H, s), 3.83 (3H, s), 6.37 (1H, d, $J = 8.3$ Hz), 6.89 (1H, d, $J = 8.3$ Hz), 6.96 (1H, br s), 9.52 (1H, br s) ppm.



3-(3-(tert-Butoxycarbonyl)amino-2-hydroxy-4-methoxyphenyl)propanol (30). Compound **29** (1.29 g, 3.96 mmol) was dissolved in THF (20 mL) and LiAlH₄ (178 mg, 4.69 mmol) was added at 0°C. After stirring for 4 hours, LiAlH₄ (100 mg, 2.64 mmol) was added again and stirred for an hour at rt. The reaction mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq. and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (CHCl₃/MeOH = 100/1) to obtain **30** (1.20 g, quant.).

HR-FD-MS (positive): Found m/z 297.1575 ($[M]^+$, calcd. for C₁₅H₂₃NO₅ 297.1576); ¹H-NMR (270 MHz, CDCl₃, rt): 1.53 (9H, s), 1.88-1.78 (2H, m), 2.74 (2H, t, J = 7.0 Hz), 3.58 (2H, t, J = 6.0 Hz), 3.84 (3H, s), 6.41 (1H, d, J = 8.3 Hz), 6.88 (1H, d, J = 8.3 Hz), 6.99 (1H, br s), 9.78 (1H, br s) ppm.

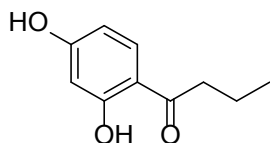


3-(3-Amino-2-hydroxy-4-methoxyphenyl)propanol (10). Compound **30** (22.9 mg, 0.077 mmol) was suspended in water (1.5 mL) and refluxed for two hours. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine, dried over sodium sulfate and evaporated to obtain **10** (14 mg, 92%).

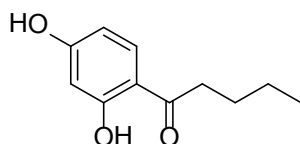
HR-FD-MS (positive): Found m/z 197.1032 ($[M]^+$, calcd. for C₁₀H₁₅NO₃ 197.1052); ¹H-NMR (270 MHz, CD₃OD, rt): 1.81-1.71 (2H, m), 2.61 (2H, t, J = 7.4 Hz), 3.53 (2H, t, J = 6.4 Hz), 3.79 (3H, s), 6.41 (1H, d, J = 8.4 Hz), 6.49 (1H, d, J = 8.4 Hz) ppm.

*General procedure for the synthesis of 4-acylresorcinol (32c-g).*²³⁾ Resorcinol (**31**, 10 mmol) was suspended in the corresponding chain length of acids (5 mL, C₄-C₈) and BF₃·Et₂O (20 mmol) was added. The mixture was stirred for 6 hours at 80°C. After cooling, water was added and the resulting

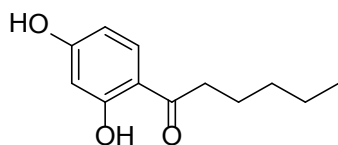
solution was extracted with EtOAc. Organic layer was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **32c-g** (83-92%).



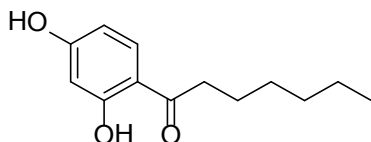
1-(2,4-Dihydroxyphenyl)butan-1-one (32c). FD-MS (positive): m/z 180 ($[M]^+$); ^1H NMR (500 MHz, acetone- d_6 , rt): 0.96 (3H, t , $J = 7.3$ Hz, H-4), 1.70 (2H, m , H-3), 2.92 (2H, t , $J = 7.3$ Hz, H-2), 6.31 (1H, d , $J = 2.3$ Hz, H-3'), 6.41 (1H, dd , $J = 8.9, 2.3$ Hz, H-5'), 7.78 (1H, d , $J = 8.9$ Hz, H-6') ppm.



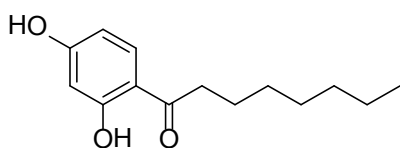
1-(2,4-Dihydroxyphenyl)pentan-1-one (32d). FD-MS (positive): m/z 194 ($[M]^+$); ^1H NMR (500 MHz, acetone- d_6 , rt): 0.92 (3H, t , $J = 7.3$ Hz, H-5), 1.38 (2H, m , H-4), 1.66 (2H, m , H-3), 2.94 (2H, t , $J = 7.4$ Hz, H-2), 6.31 (1H, d , $J = 2.4$ Hz, H-3'), 6.41 (1H, dd , $J = 8.9, 2.4$ Hz, H-5'), 7.79 (1H, d , $J = 8.9$ Hz, H-6') ppm.



1-(2,4-Dihydroxyphenyl)hexan-1-one (32e). FD-MS (positive): m/z 208 ($[M]^+$); ^1H NMR (500 MHz, acetone- d_6 , rt): 0.89 (3H, t , $J = 7.3$ Hz, H-6), 1.35 (4H, m , H-4, 5), 1.69 (2H, m , H-3), 2.94 (2H, t , $J = 7.4$ Hz, H-2), 6.31 (1H, d , $J = 2.4$ Hz, H-3'), 6.42 (1H, dd , $J = 8.9, 2.4$ Hz, H-5'), 7.80 (1H, d , $J = 8.8$ Hz, H-6') ppm.



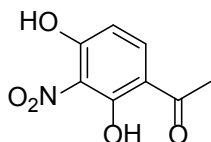
1-(2,4-Dihydroxyphenyl)heptan-1-one (32f). FD-MS (positive): m/z 222 ($[M]^+$); ^1H NMR (500 MHz, acetone- d_6 , rt): 0.88 (3H, t , $J = 6.9$ Hz, H-6), 1.31-1.41 (6H, m , H-4,5,6), 1.68 (2H, m , H-3), 2.96 (2H, t , $J = 7.4$ Hz, H-2), 6.31 (1H, d , $J = 2.4$ Hz, H-3'), 6.42 (1H, dd , $J = 8.9, 2.4$ Hz, H-5'), 7.81 (1H, d , $J = 8.9$ Hz, H-6') ppm.



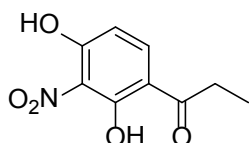
1-(2,4-Dihydroxyphenyl)octan-1-one (32g). FD-MS (positive): m/z 236 ($[M]^+$); ^1H NMR (500 MHz, acetone- d_6 , rt): 0.87 (3H, t , $J = 6.9$ Hz, H-6), 1.29-1.36 (8H, m , H-4,5,6,7), 1.69 (2H, m , H-3), 2.95 (2H, t , $J = 7.4$ Hz, H-2), 6.31 (1H, d , $J = 2.4$ Hz, H-3'), 6.42 (1H, dd , $J = 8.9, 2.4$ Hz, H-5'), 7.81 (1H, d , $J = 8.9$ Hz, H-6') ppm.

General procedure for the synthesis of 4-acyl-2-nitroresorcinol (33). Compound **32*** (10 mmol) was dissolved in acetic acid (20 mL) and the mixture of 70% nitric acid aq. (1.4 mL) and acetic acid (10 mL) was added drop by drop. After stirring for 6 hours, the reaction mixture was diluted by water and extracted with EtOAc. The organic layer was washed by water, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **33a-g** (12-27%).

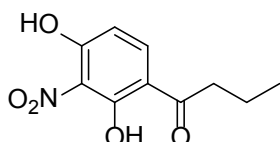
*Commercial compound was used for **32a,b**.



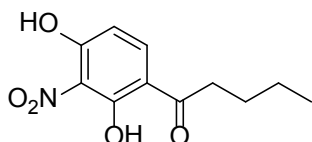
1-(2,4-Dihydroxy-3-nitrophenyl)ethan-1-one (33a). HR-FD-MS (positive): Found m/z 197.0338 ($[M]^+$, calcd. for $C_8H_7NO_5$ 197.0324); 1H NMR (500 MHz, acetone- d_6 , rt): 2.63 (3H, s , CH_3), 6.69 (1H, d , $J = 9.0$ Hz, H-5), 7.99 (1H, d , $J = 9.0$ Hz, H-6) ppm.



1-(2,4-Dihydroxy-3-nitrophenyl)propan-1-one (33b). HR-FD-MS (positive): Found m/z 211.0496 ($[M]^+$, calcd. for $C_9H_9NO_5$ 211.0481); 1H NMR (500 MHz, acetone- d_6 , rt): 1.64 (3H, t , $J = 7.2$ Hz, CH_3), 3.09 (2H, q , $J = 7.2$ Hz, CH_2), 6.69 (1H, d , $J = 9.0$ Hz, H-5), 8.0 (1H, d , $J = 9.0$ Hz, H-6) ppm.

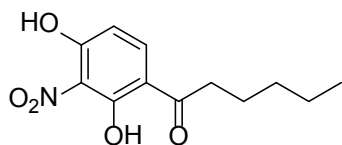


1-(2,4-Dihydroxy-3-nitrophenyl)butan-1-one (33c). HR-FD-MS (positive): Found m/z 225.0653 ($[M]^+$, calcd. for $C_{10}H_{11}NO_5$, 225.0637); 1H NMR (500 MHz, acetone- d_6 , rt): 0.98 (3H, t , $J = 7.3$ Hz, H-4), 1.73 (2H, m , H-3), 3.03 (2H, t , $J = 7.3$ Hz, H-2), 6.69 (1H, d , $J = 8.9$ Hz, H-5'), 8.03 (1H, d , $J = 8.9$ Hz, H-6') ppm.

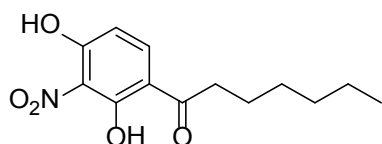


1-(2,4-Dihydroxy-3-nitrophenyl)pentan-1-one (33d). HR-FD-MS (positive): Found m/z 239.0806 ($[M]^+$, calcd. for $C_{11}H_{13}NO_5$, 239.0794); 1H NMR (500 MHz, acetone- d_6 , rt): 0.93 (3H, t , $J = 7.3$ Hz,

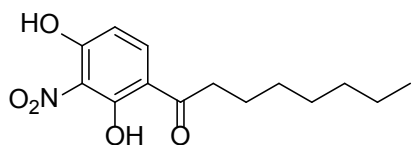
H-5), 1.41 (2H, *m*, H-4), 1.71 (2H, *m*, H-3), 3.05 (2H, *t*, *J* = 7.3 Hz, H-2), 6.69 (1H, *d*, *J* = 8.9 Hz, H-5'), 8.04 (1H, *d*, *J* = 8.9 Hz, H-6') ppm.



1-(2,4-Dihydroxy-3-nitrophenyl)hexan-1-one (33e). HR-FD-MS (positive): Found *m/z* 253.0960 ($[M]^+$, calcd. for $C_{12}H_{15}NO_5$, 253.0950); 1H NMR (500 MHz, acetone- d_6 , rt): 0.89 (3H, *t*, *J* = 7.4 Hz, H-6), 1.37 (4H, *m*, H-4,5), 1.71 (2H, *m*, H-3), 3.05 (2H, *t*, *J* = 7.4 Hz, H-2), 6.69 (1H, *d*, *J* = 9.0 Hz, H-5'), 8.04 (1H, *d*, *J* = 9.0 Hz, H-6') ppm.

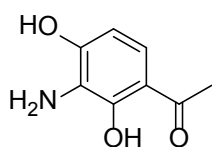


1-(2,4-Dihydroxy-3-nitrophenyl)heptan-1-one (33f). HR-FD-MS (positive): Found *m/z* 267.1118 ($[M]^+$, calcd. for $C_{13}H_{17}NO_5$, 267.1107); 1H NMR (500 MHz, acetone- d_6 , rt): 0.88 (3H, *t*, *J* = 7.4 Hz, H-6), 1.28-1.42 (6H, *m*, H-4,5,6), 1.71 (2H, *m*, H-3), 3.06 (2H, *t*, *J* = 7.4 Hz, H-2), 6.7 (1H, *d*, *J* = 9.0 Hz, H-5'), 8.04 (1H, *d*, *J* = 9.0 Hz, H-6') ppm.

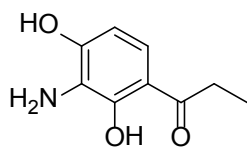


1-(2,4-Dihydroxy-3-nitrophenyl)-1-octanone (33g). HR-FD-MS (positive): Found *m/z* 281.1267 ($[M]^+$, calcd. for $C_{14}H_{19}NO_5$, 281.1263); 1H NMR (500 MHz, acetone- d_6 , rt): 0.87 (3H, *t*, *J* = 6.9 Hz, H-6), 1.28-1.36 (8H, *m*, H-4,5,6,7), 1.71 (2H, *m*, H-3), 3.06 (2H, *t*, *J* = 7.4 Hz, H-2), 6.7 (1H, *d*, *J* = 9.1 Hz, H-5'), 8.04 (1H, *d*, *J* = 9.1 Hz, H-6') ppm.

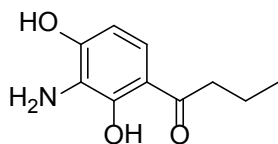
General procedure for the synthesis of 4-acyl-2-aminoresorcinol (II). Compound **33** was dissolved in MeOH and 10% Pd on charcoal was added. The mixture was stirred for 10 min under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated to give **11** (>90%) as a solid.



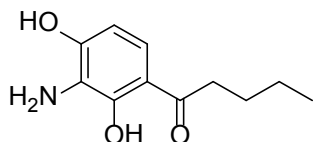
1-(3-Amino-2,4-dihydroxyphenyl)ethan-1-one (11a). HR-FD-MS (positive): Found m/z 167.0589 ($[M]^+$, calcd. for $C_8H_9NO_3$ 167.0582); 1H NMR (500 MHz, DMSO- d_6 , rt): 1.88 (3H, s , CH_3), 6.39 (1H, d , $J = 8.7$ Hz, H-5), 7.13 (1H, d , $J = 8.7$ Hz, H-6) ppm.



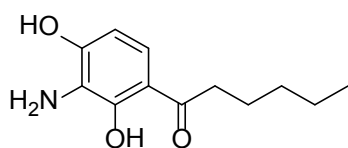
1-(3-Amino-2,4-dihydroxyphenyl)-1-propanone (11b). HR-FD-MS (positive): Found m/z 181.0730 ($[M]^+$, calcd. for $C_9H_{11}NO_3$, 181.0739); 1H NMR (500 MHz, DMSO- d_6 , rt) δ (ppm): 1.07 (3H, t , $J = 7.3$ Hz, CH_3), 2.94 (2H, q , $J = 7.3$ Hz, CH_2), 6.38 (1H, d , $J = 8.8$ Hz, H-5), 7.15 (1H, d , $J = 8.8$ Hz, H-6) ppm.



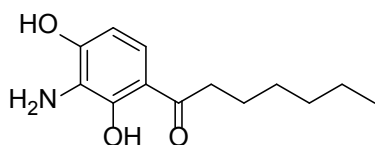
1-(3-Amino-2,4-dihydroxyphenyl)butan-1-one (11c). HR-FD-MS (positive): Found m/z 195.0869 ($[M]^+$, calcd. for $C_{10}H_{13}NO_3$, 195.0895); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.91 (3H, t , $J = 7.3$ Hz, H-4), 1.62 (2H, m , H-3), 2.87 (2H, t , $J = 7.3$ Hz, H-2), 6.38 (1H, d , $J = 8.8$ Hz, H-5'), 7.16 (1H, d , $J = 8.8$ Hz, H-6') ppm.



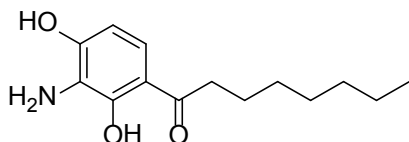
1-(3-Amino-2,4-dihydroxyphenyl)pentan-1-one (11d). HR-FD-MS (positive): Found m/z 209.1097 ($[M]^+$, calcd. for $C_{11}H_{15}NO_3$, 209.1052); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.89 (3H, t , $J = 7.3$ Hz, H-5), 1.32 (2H, m , H-4), 1.57 (2H, m , H-3), 2.87 (2H, t , $J = 7.3$ Hz, H-2), 6.35 (1H, d , $J = 8.8$ Hz, H-5'), 7.15 (1H, d , $J = 8.8$ Hz, H-6') ppm.



1-(3-Amino-2,4-dihydroxyphenyl)hexan-1-one (11e). HR-FD-MS (positive): Found m/z 223.1118 ($[M]^+$, calcd. for $C_{12}H_{17}NO_3$, 223.1208); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.85 (3H, t , $J = 7.4$ Hz, H-6), 1.29 (4H, m , H-4,5), 1.59 (2H, m , H-3), 2.87 (2H, t , $J = 7.4$ Hz, H-2), 6.35 (1H, d , $J = 8.8$ Hz, H-5'), 7.15 (1H, d , $J = 8.8$ Hz, H-6') ppm.

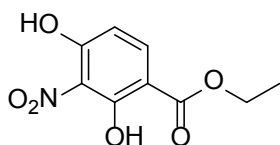


1-(3-Amino-2,4-dihydroxyphenyl)heptan-1-one (11f). HR-FD-MS (positive): Found m/z 237.1349 ($[M]^+$, calcd. for $C_{13}H_{19}NO_3$, 237.1365); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.84 (3H, t , $J = 7.4$ Hz, H-7), 1.27 (6H, m , H-4,5,6), 1.59 (2H, m , H-3), 2.88 (2H, t , $J = 7.3$ Hz, H-2), 6.37 (1H, d , $J = 8.8$ Hz, H-5'), 7.15 (1H, d , $J = 8.8$ Hz, H-6') ppm.

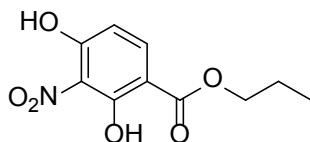


1-(3-Amino-2,4-dihydroxyphenyl)octan-1-one (11g). HR-FD-MS (positive): Found m/z 251.1484 ($[M]^+$, calcd. for $C_{14}H_{21}NO_3$, 251.1521); 1H NMR (500 MHz, $DMSO-d_6$, rt): 0.84 (3H, t , $J = 7.4$ Hz, H-8), 1.24 (8H, m , H-4,5,6,7), 1.59 (2H, m , H-3), 2.86 (2H, t , $J = 7.3$ Hz, H-2), 6.35 (1H, d , $J = 8.8$ Hz, H-5'), 7.14 (1H, d , $J = 8.8$ Hz, H-6') ppm.

General procedure for the synthesis of Alkyl 2,4-dihydroxy-3-nitrobenzoate (34b-g). Compound **34a**²⁴⁾ (1 mmol) was suspended in an alcohol (5 mL) and conc. H_2SO_4 (0.25 mL) was added. After refluxing for 24 hours, the reaction mixture was cooled, water was added and extracted by EtOAc. The organic layer was washed by water, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **34b-g** (35-83%).

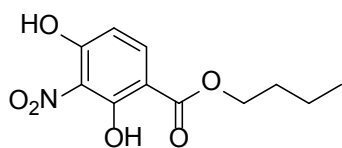


Ethyl 2,4-dihydroxy-3-nitrobenzoate (34b). HR-FD-MS (positive): Found m/z 227.0414 ($[M]^+$, calcd. for $C_9H_9NO_6$, 227.0430); 1H NMR (500 MHz, $acetone-d_6$, rt): 1.39 (3H, t , $J = 7.1$ Hz, CH_3), 4.43 (2H, q , 7.1 Hz, OCH_2), 6.71 (1H, d , $J = 9.2$ Hz, H-5), 7.88 (1H, d , $J = 9.2$ Hz, H-6) ppm.

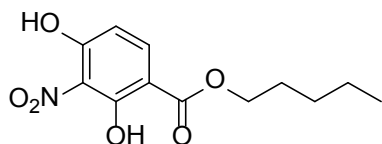


Propyl 2,4-dihydroxy-3-nitrobenzoate (34c). HR-FD-MS (positive): Found m/z 241.0607 ($[M]^+$, calcd. for $C_{10}H_{11}NO_6$, 241.0586); 1H NMR ($acetone-d_6$): 1.0 (3H, t , $J = 7.4$ Hz, CH_3), 1.79 (2H, m , CH_2), 4.31 (2H, t , $J = 6.6$ Hz, OCH_2), 6.64 (1H, d , $J = 9.0$ Hz, H-5), 7.81 (1H, d , $J = 9.0$ Hz, H-6)

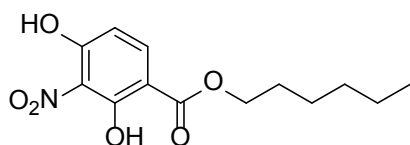
ppm.



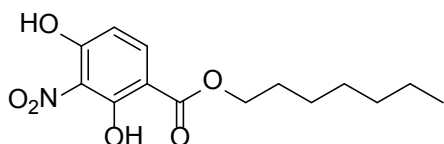
Butyl 2,4-dihydroxy-3-nitrobenzoate (34d). HR-FD-MS (positive): Found m/z 255.0751 ($[M]^+$, calcd. for $C_{11}H_{13}NO_6$, 255.0743); 1H NMR (500 MHz, acetone- d_6 , rt): 0.95 (3H, t , $J = 7.4$ Hz, CH_3), 1.46 (2H, m , CH_2), 1.75 (2H, m , CH_2), 4.36 (2H, $J = 6.6$ Hz, OCH_2), 6.63 (1H, d , $J = 9.0$ Hz, H-5), 7.8 (1H, d , $J = 9.0$ Hz, H-6) ppm.



Pentyl 2,4-dihydroxy-3-nitrobenzoate (34e). HR-FD-MS (positive): Found m/z 269.0932 ($[M]^+$, calcd. for $C_{12}H_{15}NO_6$, 269.0899); 1H NMR (500 MHz, acetone- d_6 , rt): 0.9 (3H, t , $J = 7.1$ Hz, CH_3), 1.4 (4H, m , CH_2), 1.78 (2H, m , CH_2), 4.38 (2H, $J = 6.6$ Hz, OCH_2), 6.7 (1H, d , $J = 9.0$ Hz, H-5), 7.88 (1H, d , $J = 9.0$ Hz, H-6) ppm.

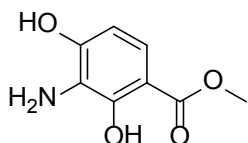


Hexyl 2,4-dihydroxy-3-nitrobenzoate (34f). HR-FD-MS (positive): Found m/z 283.1084 ($[M]^+$, calcd. for $C_{13}H_{17}NO_6$, 283.1056); 1H NMR (500 MHz, acetone- d_6 , rt): 0.89 (3H, t , $J = 7.1$ Hz, CH_3), 1.29-1.47 (6H, m , CH_2), 1.79 (2H, m , CH_2), 4.37 (2H, $J = 6.6$ Hz, OCH_2), 6.68 (1H, d , $J = 9.1$ Hz, H-5), 7.86 (1H, d , $J = 9.1$ Hz, H-6) ppm.

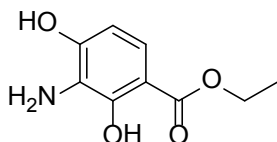


Heptyl 2,4-dihydroxy-3-nitrobenzoate (34g). HR-FD-MS (positive): Found m/z 297.1211 ($[M]^+$, calcd. for $C_{14}H_{19}NO_6$, 297.1212); 1H NMR (500 MHz, acetone- d_6 , rt): 0.86 (3H, t , $J = 7.1$ Hz, CH_3), 1.29-1.48 (8H, m , CH_2), 1.78 (2H, m , CH_2), 4.37 (2H, $J = 6.6$ Hz, OCH_2), 6.7 (1H, d , $J = 9.0$ Hz, H-5), 7.88 (1H, d , $J = 9.0$ Hz, H-6) ppm.

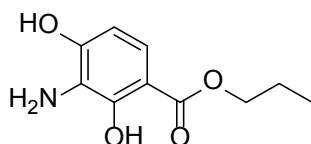
General procedure for the synthesis of Alkyl 3-amino-2,4-dihydroxybenzoate (12). Compound **34** was dissolved in MeOH and 10% Pd on charcoal was added. The mixture was stirred for 10 min under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated to give **12** (>90%) as a solid.



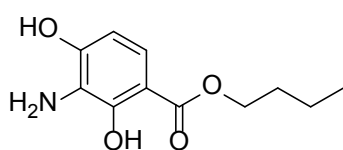
Methyl 3-amino-2,4-dihydroxybenzoate (12a). HR-FD-MS (positive): Found m/z 183.0506 ($[M]^+$, calcd. for $C_8H_9NO_4$, 183.0532); 1H NMR (500 MHz, DMSO- d_6 , rt): 3.34 (3H, s , OMe), 6.38 (1H, d , $J = 8.9$ Hz, H-5), 7.02 (1H, d , $J = 8.9$ Hz, H-6) ppm.



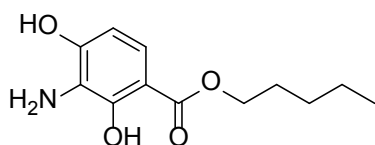
Ethyl 3-amino-2,4-dihydroxybenzoate (12b). HR-FD-MS (positive): Found m/z 197.0710 ($[M]^+$, calcd. for $C_9H_{11}NO_4$, 197.0688); 1H NMR (500 MHz, DMSO- d_6 , rt): 1.3 (3H, t , $J = 7.1$ Hz, CH_3), 4.3 (2H, q , $J = 7.1$ Hz, OCH_2), 6.37 (1H, d , $J = 8.7$ Hz, H-5), 7.03 (1H, d , $J = 8.7$ Hz, H-6) ppm.



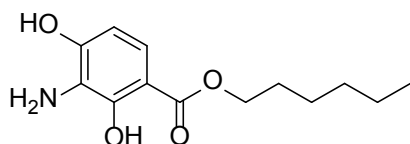
Propyl 3-amino-2,4-dihydroxybenzoate (12c). HR-FD-MS (positive): Found m/z 211.0837 ($[M]^+$, calcd. for $C_{10}H_{13}NO_4$, 211.0845); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.94 (3H, t , $J = 7.3$ Hz, CH_3), 1.68 (2H, m , CH_2), 4.17 (2H, t , $J = 6.5$ Hz, OCH_2), 6.25 (1H, d , $J = 8.7$ Hz, H-5), 6.99 (1H, d , $J = 8.7$ Hz, H-6) ppm.



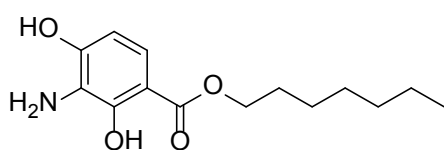
Butyl 3-amino-2,4-dihydroxybenzoate (12d). HR-FD-MS (positive): Found m/z 225.1003 ($[M]^+$, calcd. for $C_{11}H_{15}NO_4$, 225.1001); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.91 (3H, t , $J = 7.3$ Hz, CH_3), 1.39 (2H, m , CH_2), 1.66 (2H, m , CH_2), 4.23 (2H, $J = 6.5$ Hz, OCH_2), 6.30 (1H, d , $J = 8.7$ Hz, H-5), 6.99 (1H, d , $J = 8.7$ Hz, H-6) ppm.



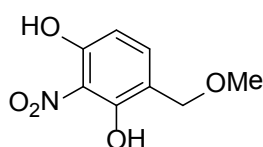
Pentyl 3-amino-2,4-dihydroxybenzoate (12e). HR-FD-MS (positive): Found m/z 239.1175 ($[M]^+$, calcd. for $C_{12}H_{17}NO_4$, 239.1158); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.88 (3H, t , $J = 7.0$ Hz, CH_3), 1.34 (4H, m , CH_2), 1.68 (2H, m , CH_2), 4.24 (2H, $J = 6.6$ Hz, OCH_2), 6.37 (1H, d , $J = 8.7$ Hz, H-5), 7.02 (1H, d , $J = 8.7$ Hz, H-6) ppm.



Hexyl 3-amino-2,4-dihydroxybenzoate (12f). HR-FD-MS (positive): Found m/z 253.1308 ($[M]^+$, calcd. for $C_{13}H_{19}NO_4$, 253.1314); 1H NMR (500 MHz, DMSO- d_6 , rt) : 0.85 (3H, *m*, CH_3), 1.28-1.36 (6H, *m*, CH_2), 1.67 (2H, *m*, CH_2), 4.23 (2H, $J = 6.4$ Hz, OCH_2), 6.34 (1H, *d*, $J = 8.7$ Hz, H-5), 7.01 (1H, *d*, $J = 8.7$ Hz, H-6) ppm.



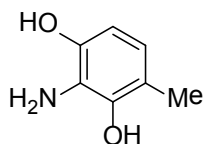
Heptyl 3-amino-2,4-dihydroxybenzoate (12g). HR-FD-MS (positive): Found m/z 267.1448 ($[M]^+$, calcd. for $C_{14}H_{21}NO_4$, 267.1471); 1H NMR (500 MHz, DMSO- d_6 , rt): 0.84 (3H, *m*, CH_3), 1.26-1.31 (8H, *m*, CH_2), 1.67 (2H, *m*, CH_2), 4.22 (2H, *t*, $J = 6.3$ Hz, OCH_2), 6.32 (1H, *d*, $J = 8.6$ Hz, H-5), 6.99 (1H, *d*, $J = 8.6$ Hz, H-6) ppm.



4-Methoxymethyl-2-nitroresorcinol (35). Compound **34a** (241.0 mg, 1.13 mmol) was dissolved in THF (15 mL) and DIPEA (1.2 mL, 7.06 mmol), MOMCl (0.4 mmol, 5.32 mmol) was added. After stirring for an hour under argon atmosphere, the reaction mixture was diluted with 1 M HCl aq. and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The obtained crude mixture of mono- and di-methoxymethylated product was then dissolved in the mixture of dry THF (13 mL) and dry Et₂O (13 mL). The solution was cooled to 0°C and LiAlH₄ (112.2 mg, 2.37 mmol) was added slowly and stirred under argon atmosphere for 20 min. The reaction mixture was quenched by the addition of methanol, diluted with 1 M HCl aq. and

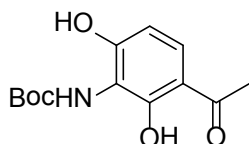
extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was dissolved in methanol (15 mL) and conc. HCl aq. (1.2 mL) was added. After stirring for 30 min at 60°C, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel chromatography (Hexane/EtOAc) to obtain **35** (175.1 mg, 79% from **34a**).

HR-FD-MS (positive): Found m/z 199.0484 ($[M]^+$, calcd. for $C_8H_9NO_5$ 197.0481); 1H -NMR (270 MHz, acetone- d_6 , rt): 3.35 (3H, s), 4.43 (2H, s), 6.63 (1H, d, $J = 8.3$ Hz), 7.51 (1H, d, $J = 8.3$ Hz) ppm; ^{13}C -NMR (67.5 MHz, acetone- d_6 , rt): 58.5, 69.3, 109.4, 119.3, 126.7, 137.8, 153.6, 155.4 ppm



2-Amino-4-methylresorcinol (13). To the solution of **35** (175.1 mg, 0.889 mmol) in methanol (18 mL), Boc_2O (0.4 mL, 1.785 mmol) and 10% Pd/C was added. The mixture was stirred overnight under hydrogen atmosphere and then passed through Celite® pad. The filtrate was evaporated and partially purified by silica-gel chromatography (Hexane/EtOAc) as the byproduct was unable to be separated from the desired product. The crude product was then dissolved in 4 M HCl in 1,4-dioxane (3.2 mL). After stirring for 30 min, the reaction mixture was evaporated and purified by preparative TLC ($CHCl_3/MeOH = 8/1$) to obtain **13** (13.1 mg, 11% from **35**).

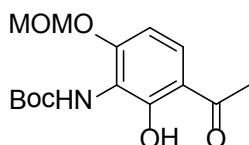
HR-FD-MS (positive): Found m/z 139.0630 ($[M]^+$, calcd. for $C_7H_9NO_2$ 139.0633); 1H -NMR (270 MHz, acetone- d_6 , rt): 2.10 (3H, s), 6.23 (1H, d, $J = 8.2$ Hz), 6.35 (1H, d, $J = 8.2$ Hz) ppm; ^{13}C -NMR (67.5 MHz, acetone- d_6 , rt): 16.0, 108.0, 117.5, 121.0, 124.4, 145.2, 145.9 ppm



N-(*tert*-Butoxycarbonyl)-3-amino-2,4-dihydroxyacetophenone (**36**).

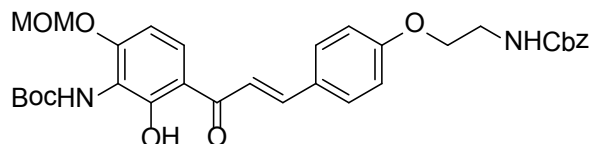
To the solution of **33a** (1.544 g, 7.831 mmol) in THF (35 mL), Boc₂O (3.325 g, 15.23 mmol) and 10% Pd/C was added and stirred overnight under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **36** (1.093 g, 52%).

HR-ESI-MS (positive): Found m/z 290.0997 ($[M+Na]^+$, calcd. for C₁₃H₁₇O₅NNa 290.0999); ¹H-NMR (270 MHz, CDCl₃, rt): 1.54 (9H, s), 2.56 (3H, s), 6.51 (1H, d, J = 9.0 Hz), 7.13 (1H, s), 7.42 (1H, d, J = 9.0 Hz), 10.96 (1H, s), 13.48 (1H, s) ppm; ¹³C-NMR (67.5 MHz, CDCl₃, rt): 26.1, 28.1, 83.2, 110.9, 112.6, 113.8, 127.4, 154.5, 154.7, 156.2, 203.0 ppm.



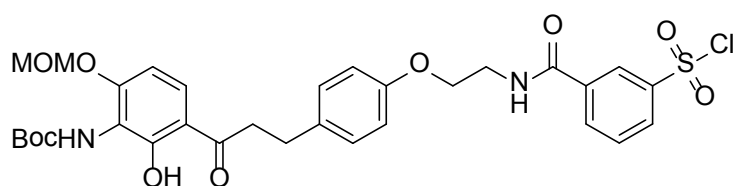
N-(*tert*-Butoxycarbonyl)-3-amino-2-hydroxy-4-(methoxymethoxy)acetophenone (**37**). To the solution of **36** (814.2 mg, 3.0 mmol) in DMF (25 mL), *i*-Pr₂NEt (0.79 mL, 4.6 mmol) and MOMCl (0.79 mL, 4.6 mmol) was added at 0°C. After stirring for 2 hours at 0°C, cold water was added and the mixture was extracted by EtOAc. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **37** (872.8 mg, 92%).

HR-ESI-MS (positive): Found m/z 334.1264 ($[M+Na]^+$, calcd. for C₁₅H₂₁O₆NNa 334.1261); ¹H-NMR (270 MHz, CDCl₃, rt): 1.49 (9H, s), 2.57 (3H, s), 3.61 (3H, s), 5.26 (2H, s), 6.71 (1H, d, J = 9.1 Hz), 7.60 (1H, d, J = 9.1 Hz), 12.81 (1H, s) ppm; ¹³C-NMR (67.5 MHz, CDCl₃, rt): 26.3, 28.2, 56.4, 80.1, 94.5, 105.5, 114.9, 115.1, 129.7, 153.6, 158.3, 159.1, 203.0 ppm.



3'-((*tert*-Butoxycarbonyl)amino)-2'-hydroxy-4'-(methoxymethoxy)-4-((2-(benzyloxycarbonyl)amino)ethoxy)chalcone (**39**). Compound **37** (742.3 mg, 2.384 mmol) and **38** (841.6 mg, 2.812 mmol) was dissolved in 1,4-dioxane (20 mL) and 50% NaOH aq. (20 mL) was added at 0°C. After stirring for 7 days under argon atmosphere, the reaction mixture was acidified by 1 M HCl aq. and extracted by EtOAc. The organic layer was washed with water and then brine. The layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **39** (554.4 mg, 39%).

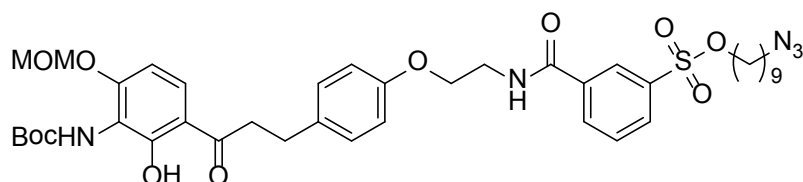
HR-ESI-MS (positive): Found m/z 615.2317 ($[M+Na]^+$, calcd. for $C_{32}H_{36}O_9N_2Na$ 615.2313); 1H -NMR (270 MHz, $CDCl_3$, rt): 1.51 (9H, s), 3.52 (3H, s), 3.63 (2H, q, $J = 4.9$ Hz), 4.09 (2H, t, $J = 4.9$ Hz), 5.12 (2H, s), 5.29 (2H, s), 5.98 (1H, s), 6.75 (1H, d, $J = 9.2$ Hz), 6.92 (2H, d, $J = 8.5$ Hz), 7.35 (5H, m), 7.44 (1H, d, $J = 15.3$ Hz), 7.59 (2H, d, $J = 8.5$ Hz), 7.77 (1H, d, $J = 9.2$ Hz), 7.85 (1H, d, $J = 15.3$ Hz), 13.58 (1H, s) ppm; ^{13}C -NMR (67.5 MHz, $CDCl_3$, rt): 28.2, 40.4, 56.5, 66.9, 67.0, 80.2, 94.5, 105.5, 114.9, 115.1, 115.4, 117.8, 127.8, 128.1, 128.2, 128.5, 128.6, 130.5, 136.2, 144.6, 153.7, 156.4, 158.2, 160.3, 160.7, 192.3 ppm.



1-(3-(*tert*-Butoxycarbonyl)amino-2-hydroxy-4-(methoxymethoxy)phenyl)-3-(4-(2-((3-(chlorosulfonyl)benzoyl)amino)ethoxy)phenyl)propan-1-one (**40**). To the solution of **39** (224.7 mg, 0.3792 mmol) in MeOH (5 mL), 10% Pd/C was added and stirred for 2.5 hours under hydrogen atmosphere. The reaction mixture was passed through Celite[®] pad and evaporated. The residue was dissolved in CH_2Cl_2 (10 mL) and pyridine (0.09 mL, 1.1 mmol), 3-(chlorosulfonyl)benzoyl chloride (0.12 mL, 0.76 mmol) was added at 0°C. After stirring for 1.5 hours under argon atmosphere, the reaction

mixture was evaporated and the residue was purified by silica-gel column chromatography (CHCl_3) to obtain **40** (125.6 mg, 50%).

HR-FD-MS (positive): Found m/z 662.1718 ($[\text{M}]^+$, calcd. for $\text{C}_{31}\text{H}_{35}\text{O}_{10}\text{N}_2\text{SCl}$ 662.1701); ^1H -NMR (270 MHz, CDCl_3 , rt): 1.49 (9H, s), 2.99 (2H, t, $J = 7.4$ Hz), 3.20 (2H, t, $J = 7.4$ Hz), 3.50 (3H, s), 3.89 (2H, q, $J = 5.0$ Hz), 4.16 (2H, t, $J = 5.0$ Hz), 5.25 (2H, s), 5.91 (1H, s), 6.68 (1H, d, $J = 9.1$ Hz), 6.85 (2H, d, $J = 8.6$ Hz), 7.15 (2H, d, $J = 8.6$ Hz), 7.59 (1H, d, $J = 9.1$ Hz), 7.72 (1H, t, $J = 7.9$ Hz), 8.18-8.14 (2H, m), 8.42 (1H, t, $J = 1.7$ Hz), 12.84 (1H, s) ppm; ^{13}C -NMR (67.5 MHz, CDCl_3 , rt): 28.0, 28.2, 29.3, 39.9, 56.5, 66.5, 77.2, 80.2, 94.5, 105.6, 114.6, 114.7, 115.0, 125.5, 128.9, 129.5, 130.2, 133.6, 133.7, 136.2, 144.7, 153.7, 156.8, 158.2, 159.2, 164.9, 204.0 ppm.

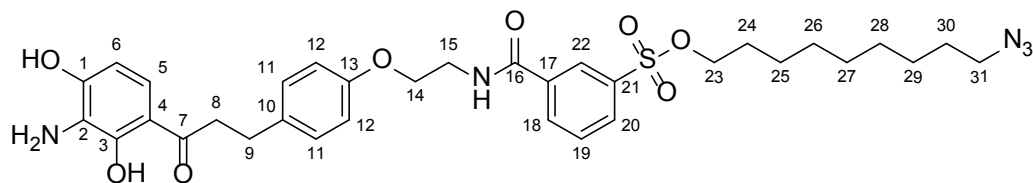


Azidononyl

3-((2-(4-(3-(3-(tert-butoxycarbonyl)amino-2-hydroxy-4-(methoxymethoxy)phenyl)-3-oxopropyl)phenoxy)ethyl)carbamoyl)benzenesulfonate (41). To the solution of **40** (772 mg, 1.17 mmol) in CH_2Cl_2 (25 mL), DMAP (281 mg, 2.32 mmol), 9-azidononanol (960 mg, 5.18 mmol) was added at 0°C . After stirring for an hour under argon atmosphere, the reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Hexane/EtOAc) to obtain **41** (307.5 mg, 33%).

HR-FD-MS (positive): Found m/z 811.3456 ($[\text{M}]^+$, calcd. for $\text{C}_{40}\text{H}_{53}\text{O}_{11}\text{N}_5\text{S}$ 811.3462); ^1H -NMR (270 MHz, CDCl_3 , rt): 1.40-1.20 (10H, m), 1.43 (9H, s), 1.70-1.50 (4H, m), 2.99 (2H, t, $J = 7.6$ Hz), 3.22 (2H, t, $J = 7.6$ Hz), 3.24 (2H, t, $J = 7.0$ Hz), 3.45 (3H, s), 3.89 (2H, q, $J = 5.1$ Hz), 4.08 (2H, t, $J = 6.5$ Hz), 4.16 (2H, t, $J = 5.1$ Hz), 5.23 (2H, s), 6.67 (1H, d, $J = 9.1$ Hz), 6.87 (2H, d, $J = 8.6$ Hz), 7.17 (2H, d, $J = 8.6$ Hz), 7.65 (1H, t, $J = 7.8$ Hz), 7.66 (1H, d, $J = 9.1$ Hz), 8.03 (1H, dt, $J = 1.7, 7.8$ Hz), 8.09 (1H, dt, $J = 1.7, 7.8$ Hz), 8.28 (1H, t, $J = 1.7$ Hz), 12.77 (1H, s) ppm; ^{13}C -NMR (67.5 MHz, CDCl_3 , rt): 25.1, 26.4, 27.6, 27.7 (3C), 28.6, 28.8, 29.0, 39.6, 39.8, 51.2, 56.1, 66.2, 71.2, 82.1, 93.9,

104.8, 114.3, 114.4, 117.2, 126.3, 129.3, 129.5, 130.2, 130.3, 132.2, 133.2, 135.5, 136.6, 151.0, 156.8, 158.1, 159.6, 165.5, 203.9 ppm.



Azidononyl

3-((2-(4-(3-(3-amino-2,4-dihydroxyphenyl)-3-oxopropyl)phenoxy)ethyl)carbamoyl)benzenesulfonate (**14**). Compound **41** (66.8 mg, 0.0823 mmol) was dissolved in 4 M HCl in 1,4-dioxane (1 mL) and stirred for 2 hours at 0°C. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (CHCl₃/MeOH) to obtain **14** (28.7 mg, 52%).

HR-ESI-MS (negative): Found m/z 666.2615 ($[M]^-$, calcd. for C₃₃H₄₀O₈N₅S 666.2603); ¹H-NMR (270 MHz, acetone-*d*₆, rt): 1.35-1.15 (10H, m, H-25,26,27,28,29), 1.65-1.45 (4H, m, H-24,30), 2.91 (2H, t, 7.6 Hz, H-9), 3.20 (2H, t, J = 7.6 Hz, H-8), 3.26 (2H, t, J = 6.8 Hz, H-31), 3.76 (2H, q, J = 5.6 Hz, H-15), 4.05 (2H, t, J = 6.4 Hz, H-23), 4.14 (2H, t, J = 5.6 Hz, H-14), 6.40 (1H, d, J = 8.8 Hz, H-6), 6.83 (2H, dd, J = 2.0, 8.6 Hz, H-12), 7.16 (2H, dd, J = 2.0, 8.6 Hz, H-11), 7.17 (1H, d, J = 8.8 Hz, H-5), 7.74 (1H, t, J = 7.9 Hz, H-19), 8.02 (1H, dt, J = 1.5, 7.9 Hz, H-20), 8.24 (1H, dt, J = 1.5, 7.9 Hz, H-18), 8.36 (1H, t, J = 1.5 Hz, H-22) ppm; ¹³C-NMR (125 MHz, acetone-*d*₆, rt): 25.9 (C-25), 27.3 (C-29), 29.4 (C-24, 26, 30), 29.6 (C-28), 29.9 (C-27), 30.2 (C-9), 40.2 (C-8), 40.3 (C-15), 51.9 (C-31), 67.0 (C-14), 72.1 (C-23), 107.6 (C-6), 113.8 (C-2), 115.3 (C-12), 120.6 (C-5), 124.3 (C-4), 127.2 (C-22), 130.2 (C-11), 130.6 (C-19), 130.9 (C-20), 133.4 (C-10, 18), 136.8 (C-21), 137.7 (C-17), 150.7 (C-1), 151.9 (C-3), 158.1 (C-13), 165.8 (C-16), 205.4 (C-7) ppm.

References

- 19) Kalisiak J, Kalisiak E, Jurczak J, *Tetrahedron*, 62, 5905-5914 (**2006**).
- 20) Hoshino Y, Okuno M, Kawamura E, Honda K, Inoue S, *Chem. Commun.*, 2281-2283 (**2009**).
- 21) Kohno Y, Ochiai K, Takita S, Kojima A, Eiraku T, Kishi T, European Patent, EP2168959 (2010/3/31).
- 22) Wang J, Lee V, Sintim HO, *Chem. Eur. J.*, 15, 2747-2750 (**2009**).
- 23) Mizobuchi S, Sato Y, *Agric. Biol. Chem.*, 49, 1327-1333 (**1985**).
- 24) Heretsch P, Giannis A, *Synthesis*, 17, 2614-2616 (**2007**).