Supporting Information

Design, synthesis, and anti-tumor activity of 4-halocolchicines and their pro-drugs activated by cathepsin B

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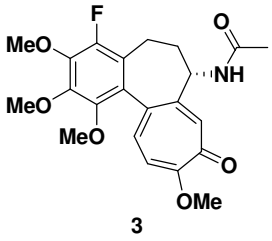
Experimental procedures for preparation and copies of ^{1}H and ^{13}C NMR spectral data of compounds **3**-**14**, **16**, **17**, **19**-**20**, **22**-**33**, procedures for *in vitro* and *in vivo* assays and the data of docking study. The purity of all compounds was confirmed more than 95% by ^{1}H- and ^{13}C-NMR spectra.

General

UV: recorded in MeOH on a JASCO V-560 instrument. IR: recorded on a JASCO FT/IR-230 spectrophotometer. ^{1}H and ^{13}C NMR spectra: recorded on JEOL JNM A-400, JNM A-500, JNM ECP-400 or JNM ECP-600 spectrometer, *J* value are given in Hz. EI-MS: direct probe insertion at 70 eV recorded on a JEOL JMS GC-mate spectrometer. FAB-MS: recorded on a JEOL JMS-AX500 or JMS-HX110 mass spectrometer. ESI-MS: recorded on a Thermo Fisher Scientific Exactive spectrometer or a JEOL JMS-T100GCV. Optical rotation: measured with a JASCO P-1020 polarimeter. Melting point: measured with a Yanagimoto Micro Melting Point Apparatus 1631A. TLC: precoated Kieselgel 60 F254 plates (Merck, 0.25 mm thick). Column chromatography: Kieselgel 60 [Merck, 70-230 mesh (for open chromatography) and 230-400 mesh (for flash chromatography)], Chromatorex NH [Fuji Silysia Chemical, 100-200 mesh (for amino-silica gel open column chromatography)]. Medium pressure liquid chromatography (MPLC): C. I. G. prepacked column CPS-HS-221-05 (Kusano Kagakukikai, SiO_2). High performance liquid chromatography (HPLC): Inertsil ODS-2 4.6 x 250 mm (GL science)

Synthesis of 4-fluorocolchicine (3).

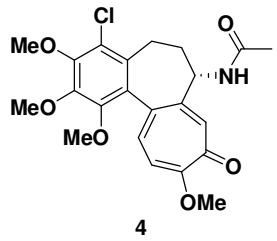


3

A mixture of NFSi (47.3 mg, 0.15 mmol) and **1** (30 mg, 0.075 mmol) in HCOOH (0.75 mL) was stirred at 70 °C under argon atmosphere for 12.5 hours. Then NFSi (47.3 mg) was added to the reaction mixture and the mixture was stirred at 70 °C for 6.5 hours. Further, NFSi (47.3 mg) was added to the mixture. After stirring at 70 °C for 6.5 hours, the reaction was quenched with saturated aqueous $Na_2S_2O_3$ at 0 °C. Saturated aqueous $NaHCO_3$ was added to the mixture and the whole mixture was extracted four times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 10 / 90) to afford 4.5 mg (15%) of **3** as a yellow oil.

4-Fluorocolchicine (3): $[\alpha]_D^{21}$: -224.8 ° (*c* = 0.02, MeOH). UV (MeOH) λ_{max} (log ε): 343 (4.17), 287 (3.62), 233 (4.42), 211 (4.36) nm. CD (MeOH, 24 °C, *c* = 0.14 mM) Δε (nm): 0 (397), + 0.97 (378), 0 (372), -7.59 (333), -5.49 (283), -13.20 (263), 0 (244), +17.94 (231), +3.11 (213). ^{1}H-NMR (400 MHz, $CDCl_3$): δ7.82 (1H, d, 6.1, -NH), 7.56 (1H, s, H-8), 7.34 (1H, d, *J* = 10.6 Hz, H-12), 6.87 (1H, d, *J* = 10.8 Hz, H-11), 4.62 (1H, ddd, *J* = 12.2, 6.2, 6.2 Hz, H-7), 4.03 (3H, s, 10-OCH_3), 4.02 (3H, s, 3-OCH_3), 4.00 (3H, s, 2-OCH_3), 3.62 (3H, s, 1-OCH_3), 3.07 (1 H, dd, *J* = 13.9, 6.0 Hz, H-5β), 2.31 (1H, dddd, *J* = 12.6, 12.6, 6.3, 6.3 Hz, H-6β), 2.05 (1H, overlapped, H-5α), 1.99 (3H, s, -NHCOC$\underline{H_3}$), 1.90 (1H, ddd, *J* = 12.2, 12.2, 6.6 Hz, H-6α). ^{13}C -NMR (100 MHz, $CDCl_3$): δ 179.5 (C-9), 170.1 (-NH$\underline{C}$OCH_3), 164.4 (C-10), 151.7 (C-7a), 149.1 (d, *J* = 242.7 Hz, C-4), 146.9 (d, *J* = 2.9 Hz, C-1), 146.1 (d, *J* = 4.6 Hz, C-2), 141.8 (d, *J* = 14.1 Hz, C-3), 135.9 (C-12), 135.4 (d, *J* = 2.5 Hz, C-12a), 130. 4 (C-8), 128.3 (d, *J* = 4.6 Hz, C-12b), 120.6 (d, *J* = 16.1 Hz, C-4a), 112.5 (C-11), 61.7 (3-OCH_3, 2-OCH_3, 1-OCH_3), 56.5 (10-OCH_3), 52.6 (C-7), 35.3 (C-6), 22.8 (-NHCO$\underline{C}H_3$), 20.6 (d, *J* = 3.3 Hz, C-5). EI-MS *m/z* (%): 417 (M^+, 44), 57 (100). HR-EI-MS: calcd for $C_{22}H_{24}FNO_6$, 417.1587; found, 417.1578.

Synthesis of 4-chlorocolchicine (4).



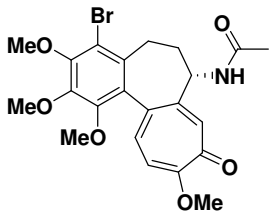
4

A mixture of NCS (133.5 mg, 1.0 mmol) and **1** (200 mg, 0.5 mmol) in AcOH (3.0 mL) was stirred at 70 °C under argon atmosphere for 3 hours. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ at 0 °C and then saturated aqueous $NaHCO_3$ was added to the mixture. The whole mixture was extracted four times with $CHCl_3$, and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 10 / 90) to afford 177.2 mg (82%) of **4** as a yellow amorphous.

4-Chlorocolchicine (4): $[\alpha]_D^{26}$: -120.7 ° (*c* = 0.06, MeOH). UV (MeOH) λ_{max} (log ε): 342 (4.08), 286 (3.46), 235 (4.38), 215 (4.30) nm. CD (MeOH, 24 °C, *c* = 0.26 mM) Δε (nm): +0.81 (381), 0 (369), -6.04 (331), -2.36 (289), -9.96 (263), 0 (250), +7.83 (237), +1.36 (217). ^{1}H-NMR (400 MHz, $CDCl_3$) δ7.60 (1H, br-d, *J* = 5.9 Hz, -NH), 7.54 (1H, s, H-8), 7.30 (1H, d, *J* = 10.6 Hz, H-12), 6.86 (1H, d, *J* = 11.0 Hz, H-11), 4.55 (1H, ddd, *J* = 12.3, 6.3, 6.3 Hz, H-7), 4.02 (3H, s, 10-OCH_3), 4.00 (3H, s, 3-OCH_3), 3.97 (3H, s, 2-OCH_3), 3.63 (3H, s, 1-OCH_3), 3.26 (1H, dd, *J* = 13.5, 4.9 Hz, H-5β), 2.30 (1H, dddd, *J* = 12.7, 12.7,

6.3, 6.3 Hz, H-6β), 2.16 (1H, ddd, *J* = 13.4, 13.4, 6.3 Hz, H-5α), 2.00 (3H, s, -NHCOC<u>H</u>$_3$), 1.85 (1H, ddd, *J* = 12.3, 12.3, 5.6 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$) δ179.4 (C-9), 170.3 (-NH<u>C</u>OCH$_3$), 164.3 (C-10), 152.2 (C-7a), 150.0 (C-4), 149.6 (C-1), 146.5 (C-2), 135.8 (C-3, C-12), 131.6 (C-12a), 130.0 (C-8), 129.7 (C-12b), 122.0 (C-4a), 112.6 (C-11), 61.44 (3-OCH$_3$), 61.39 (2-OCH$_3$), 61.0 (1-OCH$_3$), 56.4 (10-OCH$_3$), 52.7 (C-7), 34.3 (C-6), 25.7 (C-5), 22.5 (-NHCO<u>C</u>H$_3$). FAB-MS (NBA): 434 $[M+H]^+$, 436 $[M+H+2]^+$. HR-FAB-MS (NBA/PEG): calcd for $C_{22}H_{25}{}^{35}ClNO_6$ $[M+H]^+$, 434.1370; found, 434.1366; calcd for $C_{22}H_{25}{}^{37}ClNO_6$ $[M+H+2]^+$, 436.1366; found, 436.1364

Synthesis of 4-bromocolchicine (5).

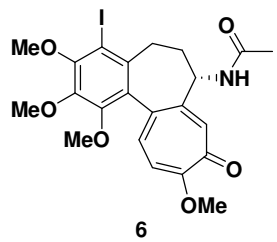


5

To a stirred solution of **1** (10.0 mg, 0.025 mmol) in AcOH (0.3 mL) was added a solution of NBS (6.8 mg, 0.038 mmol) in AcOH (0.9 mL) at room temperature under argon atmosphere and the mixuture was stirred at 70 °C for 1 hour. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ at 0 °C and then saturated aqueous $NaHCO_3$ was added to the mixture. The whole mixture was extracted four times with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel MPLC (MeOH / $CHCl_3$ = 7 / 93) to afford 11.8 mg (99%) of **5** as a yellow oil.

4-Bromocolchicine (5): $[\alpha]_D{}^{23}$: -77.3 ° (*c* = 0.14, MeOH). UV (MeOH) λ_{max} (log ε): 341 (3.87), 289 (3.46), 233 (4.21), 218 (4.18) nm. CD (MeOH, 24 °C, *c* = 0.30 mM) Δε (nm): +0.73 (380), 0 (370), -7.64 (336), -3.39 (288), -12.94 (264), 0 (250), +10.75 (230), +3.67 (217). ^{1}H-NMR (400 MHz, $CDCl_3$) δ7.86 (1H, br-d, *J* = 6.2 Hz, -NH), 7.57 (1H, s, H-8), 7.30 (1H, d, *J* = 10.8 Hz, H-12), 6.88 (1H, d, *J* = 10.8 Hz, H-11), 4.54 (1H, ddd, *J* = 12.1, 6.0, 6.0 Hz, H-7), 4.03 (3H, s, 10-OCH$_3$), 3.99 (3H, s, 3-OCH$_3$), 3.97 (3H, s, 2-OCH$_3$), 3.63 (3H, s, 1-OCH$_3$), 3.27 (1H, dd, *J* = 12.8, 4.9 Hz, H-5β), 2.33 (1H, dddd, *J* = 12.6, 12.6, 6.2, 6.2 Hz, H-6β), 2.24 (1H, ddd, *J* = 13.2, 13.2, 5.6 Hz, H-5α), 1.99 (3H, s, -NHCOC<u>H</u>$_3$), 1.85 (1H, ddd, *J* = 11.6, 11.6, 5.1 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$) δ179.5 (C-9), 170.1 (-NH<u>C</u>OCH$_3$), 164.4 (C-10), 151.6 (C-7a), 151.1 (C-4), 150.4 (C-1), 146.6 (C-2), 135.7 (C-3), 135.6 (C-12), 133.4 (C-12a), 130.2 (C-8), 130.1 (C-12b), 113.5 (C-4a), 112.3 (C-11), 61.5 (3-OCH$_3$, 2-OCH$_3$), 61.0 (1-OCH$_3$), 56.5 (10-OCH$_3$), 52.5 (C-7), 34.6 (C-6), 28.9 (C-5), 22.8 (-NHCO<u>C</u>H$_3$). FAB-MS (NBA): 478 $[M+H]^+$, 480 $[M+H+2]^+$. HR-FAB-MS (NBA/PEG): calcd for $C_{22}H_{25}{}^{79}BrNO$ $[M+H]^+{}_6$, 478.0848; found, 478.0872; calcd for $C_{22}H_{25}{}^{81}BrNO_6$ $[M+H+2]^+$, 480.0848; found, 480.0904.

Synthesis of 4-iodocolchicine (6).

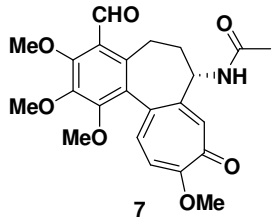


6

A mixture of NIS (112.5 mg, 0.50 mmol) and **1** (100.0 mg, 0.25 mmol) in AcOH (2.5 mL) was stirred at 70 °C under argon atmosphere for 7 hours. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ at 0 °C and then saturated aqueous $NaHCO_3$ was added to the reaction mixture. The whole mixture was extracted four times with $CHCl_3$. and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 5 / 95) to afford 129.5 mg (99%) of **6** as a brown amorphous.

4-Iodocolchicine (6): $[\alpha]_D^{20}$: -46.7 ^{o} (*c* = 0.05, MeOH). UV (MeOH) λ_{max} (log ε): 342 (4.07), 289 (3.55), 235 (4.45) nm. CD (MeOH, 24 ^{o}C, *c* = 0.17 mM) Δε (nm): +0.77 (377), 0 (369), -4.75 (333), -3.50 (305), -7.89 (266), 0 (253), +4.17 (245), +2.49 (236), +9.76 (225), +3.45 (215). ^{1}H-NMR (400 MHz, $CDCl_3$): δ7.85 (1H, br-s, -NH), 7.58 (1H, s, H-8), 7.29 (1H, d, *J* = 11.4 Hz, H-12), 6.88 (1H, d, *J* = 10.8 Hz, H-11), 4.51 (1H, ddd, *J* = 12.0, 6.0, 6.0 Hz, H-7), 4.03 (3H, s, 10-OCH_3), 3.97 (3H, s, 3-OCH_3), 3.95 (3H, s, 2-OCH_3), 3.62 (3H, s, 1-OCH_3), 3.18 (1H, dd, *J* = 13.3, 5.0 Hz, H-5β), 2.42 (1H, ddd, *J* = 13.4, 13.4, 5.9 Hz, H-5α), 2.31 (1H, dddd, *J* = 12.3, 12.3, 6.1, 6.1 Hz, H-6β), 1.99 (3H, s, -NHCOC<u>H</u>$_3$), 1.80 (1H, ddd, *J* = 12.1, 12.1, 5.7 Hz, H-6α). ^{13}C-NMR (100MHz, $CDCl_3$): δ179.5 (C-9), 170.2 (-NH<u>C</u>OCH_3), 164.4 (C-10), 153.4 (C-7a), 151.7 (C-4), 151.4 (C-1), 145.6 (C-2), 136.7 (C-3), 136.2 (C-12), 135.6 (C-12a), 130.2 (C-8), 129.6 (C-12b), 112.4 (C-11), 92.1 (C-4a), 61.5 (3-OCH_3), 61.4 (2-OCH_3), 60.8 (1-OCH_3), 56.5 (10-OCH_3), 52.5 (C-7), 34.5 (C-6), 29.6 (C-5), 22.8 (-NHCO<u>C</u>H_3). EI-MS *m/z* (%): 525 (M^+, 69), 438 (100). HR-EI-MS: calcd for $C_{22}H_{24}INO_6$, 525.0648; found, 525.0661.

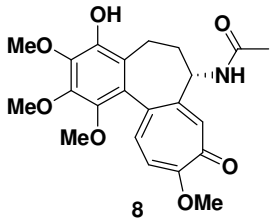
Synthesis of 4-formylcolchicine (7).



To a stirred solution of **1** (100.0 mg, 0.025 mmol) in CH_2Cl_2 (2.5 mL) were added $SnCl_4$ (87.8 μL, 0.75 mmol) and dichloromethyl methylether (0.3 mL, 3.0 mmol) under argon atmosphere at 0 ^{o}C. After stirring for 30 minutes at 0 ^{o}C, the reaction mixture was warmed to room temperature and stirred for 12 hours and 30 minutes. The reaction was quenched by adding ice. The mixture was diluted with $CHCl_3$ and was stirred for 1 hour. After separation of the layers, the organic layer was washed with 10 % aqueous NaOH and then H_2O, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / AcOEt = 10 / 90) to afford 134.4 mg (quant.) of **7** as a yellow oil.

4-Formylcolchicine (7): $[\alpha]_D^{25}$: -76.9 ^{o} (*c* = 0.09, MeOH). UV (MeOH) λ_{max} (log ε): 341 (2.96), 299 (2.57), 244 (3.23) nm. CD (MeOH, 24 ^{o}C, *c* = 0.30 mM) Δε (nm): +0.28 (381), 0 (375), -6.77 (334), -3.41 (290), -10.48 (264), 0 (252), +7.24 (242), +0.15 (215). ^{1}H-NMR (400 MHz, $CDCl_3$): δ10.46 (1H, s, -C<u>H</u>O), 7.55 (1H, br-d, *J* = 6.6 Hz, -NH), 7.52 (1H, s, H-8), 7.23 (1H, d, *J* = 10.8 Hz, H-12), 6.85 (1H, d, *J* = 10.8 Hz, H-11), 4.54 (1H, ddd, *J* = 12.3, 6.2, 6.2 Hz, H-7), 4.06 (3H, s, 10-OCH_3), 4.02 (3H, s, 3-OCH_3), 3.98 (3H, s, 2-OCH_3), 3.87 (1H, dd, *J* = 13.3, 4.7 Hz, H-5β), 3.69 (3H, s, 1-OCH_3), 2.34 (1H, dddd, *J* = 12.7, 12.7, 6.4, 6.4 Hz, H-6β), 2.01 (3H, s, -NHCOC<u>H</u>$_3$), 2.00 (1H, ddd, *J* = 13.2, 13.2, 6.7 Hz, H-5α), 1.81 (1H, ddd, *J* = 12.2, 12.2, 4.9 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ192.0 (-CHO), 179.5 (C-9), 170.2 (-NH<u>C</u>OCH_3), 164.4 (C-10), 158.0 (C-7a), 155.7 (C-4), 151.7 (C-1), 145.2 (C-2), 136.3 (C-3), 135.7 (C-12), 135.3 (C-12a), 130.7 (C-8), 130.1 (C-12b), 123.0 (C-4a), 112.2 (C-11), 62.4 (3-OCH_3), 61.5 (2-OCH_3), 61.4 (1-OCH_3), 56.5 (10-OCH_3), 52.6 (C-7), 35.3 (C-6), 24.0 (C-5), 22.8 (-NHCO<u>C</u>H_3). IR ν_{max} (ATR) cm^{-1}: 3264, 2927, 2849, 1679, 1556. FAB-MS (NBA): 428 $[M+H]^+$. HR-FAB-MS (NBA/PEG): calcd for $C_{23}H_{26}NO_7$ $[M+H]^+$, 428.1709; found, 428.1723.

Synthesis of 4-hydroxycolchicine (8).

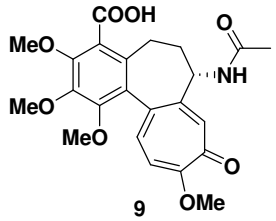


8

To a stirred solution of **7** (15.7 mg, 0.037 mmol) in MeOH (0.37 mL) was added 80% MMPP (45.8 mg, 0.074 mmol) under argon atmosphere and the mixture was stirred at room temperature for 4 hours. The reaction was quenched by adding saturated aqueous $NaHCO_3$ and then 1*N* HCl was added to the mixture. The whole mixture was extracted four times with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 3 / 97) to afford 5.7 mg (37%) of **8** as a yellow oil.

4-Hydroxycolchicine (8): $[\alpha]_D^{23}$: -164.4 ^{o} (*c* = 0.08, MeOH). UV (MeOH) λ_{max} (log ε): 347 (4.11), 287 (3.44), 234 (4.37), 222 (4.32) nm. CD (MeOH, 24 ^{o}C, *c* = 0.27 mM) Δε (nm): +0.72 (383), 0 (377), -9.06 (337), -1.80 (282), -11.08 (262), 0 (247), +10.82 (230), +3.54 (217). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.46 (1H, s, H-8), 7.32 (1H, d, *J* = 10.6 Hz, H-12), 6.86 (1H, br-d, *J* = 8.4 Hz, -NH), 6.83 (1H, d, *J* = 11.2 Hz, H-11), 5.68 (1H, br-s, -OH), 4.63 (1H, ddd, *J* = 12.3, 6.3, 6.3 Hz, H-7), 4.02 (3H, s, 10-OCH_3), 4.00 (3H, s, 3-OCH_3), 3.99 (3H, s, 2-OCH_3), 3.58 (3H, s, 1-OCH_3), 3.15 (1H, dd, *J* = 13.7, 5.9 Hz, H-5β), 2.25 (1H, dddd, *J* = 12.6, 12.6, 6.3, 6.3 Hz, H-6β), 2.00 (3H, s, -NHCOC$\underline{H}_3$), 1.97 (1H, ddd, *J* = 13.4, 13.4, 6.6 Hz, H-5α), 1.78 (1H, ddd, *J* = 11.9, 11.9, 5.9 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.5 (C-9), 170.0 (-NH$\underline{C}OCH_3$), 164.1 (C-10), 152.1 (C-7a), 144.8 (C-4), 143.8 (C-1), 141.8 (C-2), 139.9 (C-3), 136.3 (C-12), 135.6 (C-12a), 130.4 (C-8), 129.1 (C-12b), 118.9 (C-4a), 112.6 (C-11), 61.7 (3-OCH_3), 61.3 (2-OCH_3), 61.2 (1-OCH_3), 56.4 (10-OCH_3), 52.7 (C-7), 35.4 (C-6), 22.9 (-NHCO$\underline{C}H_3$), 21.0 (C-5). FAB-MS (NBA): 416 $[M+H]^+$. HR-FAB-MS (NBA/PEG): calcd for $C_{22}H_{26}NO_7$ $[M+H]^+$, 416.1709; found, 416.1697.

Synthesis of colchicine-4-carboxylic acid (9).



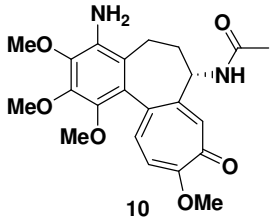
9

To a stirred solution of **7** (170 mg, 0.4 mmol) in *t*-BuOH (2.0 mL) and H_2O (1.0 mL) were added 2-methyl-2-butene (2M in THF) (3 mL, 6.0 mmol), $NaClO_2$ (181 mg, 2.0 mmol), and NaH_2PO_4 (480 mg, 4.0 mmol) under argon atmosphere. After stirring for 3 hours at room temperature, the reaction was quenched by adding saturated aqueous NH_4Cl. The whole mixture was extracted four times with 10% MeOH / $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (10% AcOH in MeOH / $CHCl_3$ = 10 / 90) to afford 174.6 mg (99%) of **9** as a white amorphous.

Colchicine-4-carboxylic acid (9): $[\alpha]_D^{25}$: -132.6 ^{o} (*c* = 0.12, MeOH). UV (MeOH) λ_{max} (log ε): 344 (4.15), 286 (3.53), 237 (4.43), 211 (4.34) nm. CD (MeOH, 24 ^{o}C, *c* = 0.28 mM) Δε (nm): +0.59 (382), 0 (373), -7.72 (334), -3.74 (290), -13.36 (264), 0 (250), +2.80 (245), 2.17 (241), +8.83 (228), 0 (215). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65 (1H, s, H-8), 7.44 (1H, br-s, -NH), 7.32 (1H, d, *J* = 10.8 Hz, H-12), 6.92 (1H, d, *J* = 11.0 Hz, H-11), 4.64 (1H, ddd, *J* = 12.2, 6.3, 6.3 Hz, H-7), 4.03 (3H, s, 10-OCH_3), 3.99 (3H, s, 3-OCH_3), 3.96 (3H, s, 2-OCH_3), 3.65 (3H, s, 1-OCH_3), 2.81 (1H, dd, *J* = 13.7, 4.6 Hz, H-5β), 2.33 (1H, dddd, *J* = 12.4, 12.4, 6.2, 6.2 Hz, H-6β), 2.18, (1H, ddd, *J* = 13.1, 13.1, 6.2 Hz, H-5α), 1.99 (3H, s, -NHCOC$\underline{H}_3$), 1.81 (1H, ddd, *J* = 11.6, 11.6, 5.6 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.5 (C-9),

176.4 (-COOH), 171.2 (-NHCOCH$_3$), 170.3 (C-10), 164.3 (C-7a), 152.7 (C-4), 152.1 (C-1), 150.5 (C-2), 145.5 (C-3), 136.3 (C-12), 130.9 (C-12a), 130.2 (C-8), 129.4 (C-12b), 124.0 (C-4a), 113.4 (C-11), 61.8 (3-OCH$_3$), 61.5 (2-OCH$_3$), 61.2 (1-OCH$_3$), 56.4 (10-OCH$_3$), 52.6 (C-7), 35.6 (C-6), 26.4 (-NHCOCH$_3$), 20.8 (C-5). IR ν_{max} (ATR) cm^{-1}: 3260, 2926, 2849, 1716, 1541. EI-MS *m/z* (%): 443 (M$^+$, 45), 370 (80), 338 (100). HR-ESI-MS: calcd for $C_{23}H_{26}NO_8$ [M+H]$^+$, 444.1636; found, 444.1653.

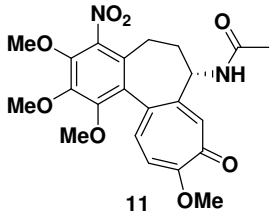
Synthesis of 4-aminocolchicine (10).



To a stirred solution of **9** (5.0 mg, 0.011 mmol) in THF (0.3 mL) were added Et_3N (2.3 μL, 0.017mmol) and DPPA (2.8 μL, 0.013 mmol) under argon atmosphere at 0 °C and the mixture was stirred for 4 hours at room temperature. After addition of H_2O (0.15 mL), the mixture was refluxed for 1 hour. The reaction was quenched by adding saturated aqueous K_2CO_3 and the whole mixture was extracted four times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel MPLC (MeOH / $CHCl_3$ = 10 / 90) to afford 1.7 mg (37%) of **10** as a yellow oil.

4-Aminocolchicine (10): $[\alpha]_D^{20}$: -136.2 ° (*c* = 0.09, MeOH). UV (MeOH) λ_{max} (log ε): 347 (4.14), 289 (3.63), 237 (4.49) nm. CD (MeOH, 24 °C, *c* = 0.164 mM) Δε (nm): +0.20 (395), 0 (388), -8.95 (343), 0 (292), +0.39 (290), 0 (283), -9.57 (263), 0 (250), +1.67 (246), +0.47 (241), +17.27 (225), +9.04 (213). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.49 (1H, s, H-8), 7.33 (1H, d, *J* = 10.8 Hz, H-12), 7.09 (1H, br-s, -NH), 6.85 (1H, d, *J* = 10.8 Hz, H-11), 4.65 (1H, ddd, *J* = 12.1, 6.1, 6.1 Hz, H-7), 4.00 (3H, s, 10-OCH$_3$), 3.99 (3H, s, 3-OCH$_3$), 3.94 (3H, s, 2-OCH$_3$), 3.72 (2H, br-s, 4-NH$_2$), 3.55 (3H, s, 1-OCH$_3$), 2.63 (1H, dd, *J* = 14.0, 5.1 Hz, H-5β), 2.26 (1H, dddd, *J* = 12.6, 12.6, 6.3, 6.3 Hz, H-6β), 2.12, (1H, ddd, *J* = 13.9, 13.9, 6.1 Hz, H-5α), 2.00 (3H, s, -NHCOCH$_3$), 1.80 (1H, ddd, *J* = 11.7, 11.7, 5.6 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.5 (C-9), 170.0 (-NHCOCH$_3$), 164.0 (C-10), 152.1 (C-7a), 145.5 (C-4), 143.0 (C-1), 141.3 (C-2), 136.8 (C-3), 135.7 (C-12), 133.0 (C-12a), 130.3 (C-8), 129.1 (C-12b), 117.9 (C-4a), 112.7 (C-11), 61.6 (3-OCH$_3$), 61.2 (2-OCH$_3$), 60.5 (1-OCH$_3$), 56.4 (10-OCH$_3$), 52.7 (C-7), 35.0 (C-6), 22.9 (-NHCOCH$_3$), 22.7 (C-5). FAB-MS (NBA): 415 [M+H]$^+$. HR-FAB-MS (NBA): calcd for $C_{22}H_{27}N_2O_6$ [M+H]$^+$, 415.1869; found, 415.1857.

Synthesis of 4-nitrocolchicine (11).

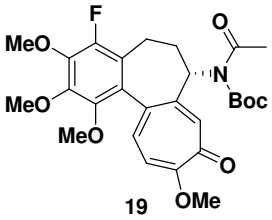


To a stirred solution of CAN (Cerium(IV)ammonium nitrate) (14.4 mg, 0.026 mmol) in CH_2Cl_2 (0.3 mL) were added **1** (10.0 mg, 0.025 mmol) and TFAA (Trifluoroacetic anhydrate) (12.2 μL, 0.088mmol) under argon atmosphere at 0 °C. After stirring for 16 hours at room temperature, the reaction was quenched by adding saturated aqueous $NaHCO_3$ and the whole mixture was extracted four times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel MPLC (MeOH / AcOEt = 10 / 90) and silica gel MPLC (MeOH / $CHCl_3$ = 5 / 95) to afford 1.6 mg (15%) of **11** as a yellow oil.

4-Nitrocolchicine (11): $[\alpha]_D^{20}$: -165.4 ° (*c* = 0.05, MeOH). UV (MeOH) λ_{max} (log ε): 341 (4.05), 289 (3.47), 233 (4.30), 214 (4.24) nm. CD (MeOH, 24 °C, *c* = 0.14 mM) Δε (nm): -7.05 (340), -1.40 (287),

-1.79 (283), -1.38 (279), -8.42 (262), 0 (246), +10.07 (231), -2.23 (214). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.47 (1H, s, H-8), 7.25 (1H, d, J = 11.0 Hz, H-12), 7.19 (1H, br-s, -NH), 6.84 (1H, d, J = 10.8 Hz, H-11), 4.61 (1H, ddd, J = 12.1, 6.1, 6.1 Hz, H-7), 4.04 (3H, s, 10-OCH_3), 4.03 (3H, s, 3-OCH_3), 4.01 (3H, s, 2-OCH_3), 3.68 (3H, s, 1-OCH_3), 2.56 (1H, dd, J = 13.6, 5.0 Hz, H-5β), 2.34 (1H, dddd, J = 12.8, 12.8, 6.4, 6.4 Hz, H-6β), 2.33, (1H, ddd, J = 13.3, 13.3, 6.6 Hz, H-5α), 2.02 (3H, s, -NHCOC$\underline{H}_3$), 1.83 (1H, ddd, J = 12.0, 12.0, 6.0 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.4 (C-9), 170.1 (-NH$\underline{C}$OCH_3), 164.7 (C-10), 152.9 (C-7a), 150.6 (C-4), 146.2 (C-1), 145.4 (C-2), 141.3 (C-3), 135.9 (C-12), 134.1 (C-12a), 130.5 (C-8), 129.4 (C-12b), 125.7 (C-4a), 112.0 (C-11), 61.6 (3-OCH_3), 61.4 (2-OCH_3), 56.7 (1-OCH_3), 56.4 (10-OCH_3), 52.5 (C-7), 35.1 (C-6), 24.6 (C-5), 22.8 (-NHCO$\underline{C}H_3$). EI-MS *m/z* (%): 444 (M^+, 36), 57 (100). HR-ESI-MS: calcd for $C_{22}H_{24}N_2O_8Na$ $[M+Na]^+$, 467.14160; found, 467.14303.

Synthesis of *N*-Boc-4-fluorocolchicine (19).

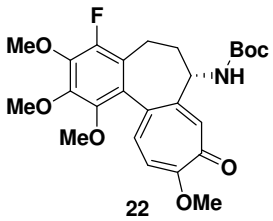


19

To a stirred solution of **3** (12 mg, 0.029 mmol) in CH_3CN (0.5 mL) were added DMAP (3.5 mg, 0.029 mmol), Et_3N (12 μM, 0.087 mmol), and Boc_2O (33.3 μM, 0.145 mmol) under argon atmosphere. After refluxing for 7 hours and 30 minutes, the reaction mixture was diluted with $CHCl_3$ and the whole mixture was washed three times with saturated aqueous citric acid. Obtained aqueous layers were extracted three times with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / AcOEt = 1 / 99) to afford 12.2 mg (81%) of **19** as yellow amorphous.

***N*-Boc-4-fluorocolchicine (19)**: $[\alpha]_D^{20}$: -123.4 ^{o} (c = 0.02, MeOH). UV (MeOH) λ_{max} (log ε): 343 (4.30), 286 (3.74), 243 (4.53), 232 (4.53), 222 (4.51) nm. CD (MeOH, 24 ^{o}C, c = 0.23 mM) Δε (nm): 0 (382), -10.66 (334), -5.92 (287), -17.22 (263), 0 (249), +50.53 (231), +4.09 (209). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, s, H-8), 7.19 (1H, d, J = 10.6 Hz, H-12), 6.75 (1H, d, J = 11.0 Hz, H-11), 5.11 (1H, dd, J = 12.5, 5.9 Hz, H-7), 4.00 (3H, s, 10-OCH_3), 3.99 (3H, s, 3-OCH_3), 3.98 (3H, s, 2-OCH_3), 3.62 (3H, s, 1-OCH_3), 3.13 (1H, dd, J = 13.9, 6.0 Hz, H-5β), 2.66 (1H, ddd, J = 11.9, 11.9, 6.9 Hz, H-5α), 2.28 (3H, s, -NHCOC$\underline{H}_3$), 2.11 (1H, ddd, J = 13.4, 13.4, 7.3 Hz, H-6α), 1.95 (1H, dddd, J = 12.3, 12.3, 6.2, 6.2 Hz, H-6β), 1.57 (9H, s, -C(C$\underline{H}_3$)$_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.3 (C-9), 171.1 (-N$\underline{C}$OCH_3), 164.2 (-N$\underline{C}$OOC$(CH_3)_3$), 153.3 (C-10), 148.8 (d, J = 241.8 Hz, C-4), 148.4 (C-7a), 146.9 (d, J = 3.1 Hz, C-1), 146.0 (d, J = 4.2 Hz, C-2), 141.6 (d, J = 14.2 Hz, C-3), 134.7 (C-12), 134.4 (d, J = 1.9 Hz, C-12a), 132.8 (C-8), 129.0 (d, J = 4.6 Hz, C-12b), 120.3 (d, J = 16.1 Hz, C-4a), 111.3 (C-11), 84.8 (-NCOO$\underline{C}(CH_3)_3$), 61.6 (1-OCH_3, 2-OCH_3), 61.5 (3-OCH_3), 57.6 (C-7), 56.2 (10-OCH_3), 31.3 (C-6), 27.8 (-NCOOC($\underline{C}H_3$)$_3$), 25.5 (-NHCO$\underline{C}H_3$), 20.8 (d, J = 3.8 Hz, C-5). EI-MS *m/z* (%): 517 (M^+, 19), 417 (100). HR-EI-MS: calcd for $C_{27}H_{32}NO_8F$, 517.2112; found, 517.2118.

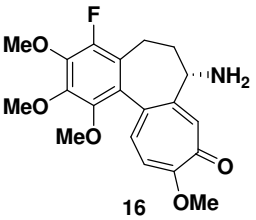
Synthesis of *N*-Boc-4-fluorodeacetylcolchicine (22).



To a stirred solution of **19** (157.4 mg, 0.30 mmol) in MeOH (3 mL) was added 1M NaOMe in MeOH (0.6 mL, 0.6 mmol) under argon atmosphere at 0 °C. After stirring for 1 hour at room temperature, the reaction was quenched with saturated aqueous NaCl and the whole mixture was extracted four times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 1 / 99) to afford 130.3 mg (91%) of **22** as yellow amorphous.

***N*-Boc-4-fluorodeacetylcolchicine (22)**: $[\alpha]_D^{20}$: -168.3 ° (*c* = 0.05, MeOH). UV (MeOH) λ_{max} (log ε): 343 (4.04), 288 (3.49), 233 (4.28), 216 (4.23). CD (MeOH, 24 °C, *c* = 0.20 mM) Δε (nm): +0.73 (379), 0 (372), -6.09 (336), -3.71 (300), -10.40 (264), 0 (246), +17.22 (231), +1.21 (207). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.51 (1H, s, H-8), 7.26 (1H, d, *J* = 10.7 Hz, H-12), 6.80 (1H, d, *J* = 11.0 Hz, H-11), 5.06 (1H, br-d, *J* = 7.6 Hz, -NH), 4.38 (1H, ddd, *J* = 12.4, 6.3, 6.3 Hz, H-7), 4.01 (3H, s, 10-OCH_3), 4.00 (3H, s, 3-OCH_3), 3.99 (3H, s, 2-OCH_3), 3.61 (3H, s, 1-OCH_3), 3.04 (1H, dd, *J* = 13.8, 6.0 Hz, H-5β), 2.26 (1H, dddd, *J* = 12.6, 12.6, 6.3, 6.3 Hz, H-6β), 2.02 (1H, ddd, *J* = 13.5, 13.5, 6.7 Hz, H-5α), 1.69 (1H, ddd, *J* = 11.8, 11.8, 6.5 Hz, H-6α), 1.37 (9H, s, -C($C\underline{H}_3)_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.4 (C-9), 164.2 (-N$\underline{C}$OOC$(CH_3)_3$), 154.3 (C-10), 150.6 (C-7a), 148.9 (d, *J* = 241.9 Hz, C-4), 146.8 (d, *J* = 2.5 Hz, C-1), 146.0 (d, *J* = 5.0 Hz, C-2), 141.6 (d, *J* = 13.7 Hz, C-3), 135.3 (C-12), 134.6 (C-12a), 131.2 (C-8), 128.3 (d, *J* = 4.6 Hz, C-12b), 120.6 (d, *J* = 16.5 Hz, C-4a), 111.7 (C-11), 79.9 (-NCOO$\underline{C}(CH_3)_3$), 61.7 (1-OCH_3), 61.6 (2-OCH_3, 3-OCH_3), 56.3 (10-OCH_3), 52.9 (C-7), 36.4 (C-6), 28.2 (-NCOOC($\underline{C}H_3)_3$), 20.7 (d, *J* = 3.3 Hz, C-5). EI-MS *m/z* (%): 475 (M^+, 19), 419 (100). HR-EI-MS: calcd for $C_{25}H_{30}NO_7F$, 475.2006; found, 475.2007.

Synthesis of 4-fluorodeacetylcolchicine (16).

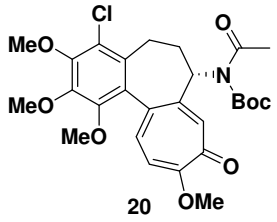


22 (65.0 mg, 0.14 mmol) was dissolved in TFA (0.2 mL, 2.66 mmol) under argon atmosphere at 0 °C. After stirring for 40 minutes at room temperature, the reaction was quenched with 5*N* NaOH and the whole mixture was extracted three times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel MPLC (MeOH / $CHCl_3$ = 10 / 90) to afford 55.0 mg (quantitative) of **16** as yellow amorphous.

4-Fluorodeacetylcolchicine (16): $[\alpha]_D^{20}$: -70.7 ° (*c* = 0.07, MeOH). UV (MeOH) λ_{max} (log ε): 344 (3.88), 289 (3.37), 232 (4.12), 212 (4.07) nm. CD (MeOH, 24 °C, *c* = 0.14 mM) Δε (nm): +0.47 (385), 0 (372), -2.75 (300), -6.97 (261), 0 (248), +17.28 (230), +0.17 (205). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, s, H-8), 7.19 (1H, d, *J* = 10.6 Hz, H-12), 6.79 (1H, d, *J* = 10.8 Hz, H-11), 4.01 (3H, s, 10-OCH_3), 4.00 (3H, s, 3-OCH_3), 3.97 (3H, s, 2-OCH_3), 3.69 (1H, dd, *J* = 10.9, 6.3 Hz, H-7), 3.59 (3H, s, 1-OCH_3), 3.00 (1H, dd, *J* = 13.7, 6.2 Hz, H-5β), 2.29 (1H, dddd, *J* = 12.8, 12.8, 6.4, 6.4 Hz, H-6β), 2.02 (1H, ddd, *J* = 13.5, 13.5, 6.9 Hz, H-5α), 1.59 (1H, ddd, *J* = 11.7, 11.7, 7.0 Hz, H-6α). ^{13}C-NMR (125MHz, $CDCl_3$): δ 179.6 (C-9), 164.1 (C-10), 153.7 (C-7a), 148.9 (d, *J* = 242.4 Hz, C-4), 146.5 (d, *J* = 2.7 Hz, C-1), 145.6 (d, *J* = 5.0 Hz, C-2), 141.7 (d, *J* = 13.7 Hz, C-3), 135.0 (d, *J* = 2.3 Hz, C-12a), 134.8 (C-12), 131.8 (C-8), 128.3 (d, *J* = 5.0 Hz, C-12b), 121.6 (d, *J* = 16.0 Hz, C-4a), 111.5 (C-11), 61.7 (d, *J* = 4.6 Hz, 3-OCH_3), 61.5

(2-OCH_3), 61.1 (1-OCH_3), 56.3 (10-OCH_3), 53.6 (C-7), 39.2 (C-6), 21.3 (d, *J* = 3.7 Hz, C-5). EI-MS *m/z* (%): 375 (M^+, 68), 83 (100). HR-ESI-MS: calcd for $C_{20}H_{22}NO_5FNa$ $[M+Na]^+$, 398.13664; found, 398.13797.

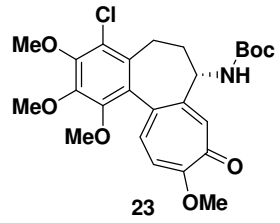
Synthesis of *N*-Boc-4-chlorocolchicine (20).



According to the synthetic procedure from **3** to **19** described above, **20** (248.2 mg, 95%) was obtained as yellow amorphous from **4** (214 mg, 0.49 mmol).

***N*-Boc-4-chlorocolchicine (20)**: $[\alpha]_D^{20}$: -10.4 ^{o} (*c* = 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 340 (4.14), 286 (3.58), 233 (4.44), 214 (4.39) nm. CD (MeOH, 24 ^{o}C, *c* = 0.17 mM) Δε (nm): 0 (380), -7.40 (331), -3.38 (287), -11.66 (264), 0 (253), +28.19 (233), +1.43 (214). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.53 (1H, s, H-8), 7.24 (1H, d, *J* = 10.6 Hz, H-12), 6.74 (1H, d, *J* = 10.8 Hz, H-11), 5.06 (1H, dd, *J* = 11.9, 6.1 Hz, H-7), 3.98 (6H, s, 10-OCH_3, 3-OCH_3), 3.96 (3H, s, 2-OCH_3), 3.62 (3H, s, 1-OCH_3), 3.32 (1H, dd, *J* = 13.5, 5.6 Hz, H-5β), 2.63 (1H, ddd, *J* = 11.3, 11.3, 7.0 Hz, H-5α), 2.30 (3H, s, -NHCOC$\underline{H_3}$), 2.25 (1H, overlapped, H-6α), 1.94 (1H, dddd, *J* = 12.5, 12.5, 6.3, 6.3 Hz, H-6β), 1.57 (9H, s, -C(C$\underline{H_3}$)$_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* 179.3 (C-9), 164.2 (-N$\underline{C}$OCH_3), 153.3 (-N$\underline{C}$OOC$(CH_3)_3$), 150.0 (C-10), 149.8 (C-7a), 148.2 (C-4), 146.7 (C-1), 134.8 (C-2), 134.5 (C-3), 134.5 (C-12), 132.8 (C-12a), 131.5 (C-8), 130.6 (C-12b), 121.9 (C-4a), 111.3 (C-11), 84.9 (-NCOO$\underline{C}$$(CH_3)_3$), 61.6 (3-$OCH_3$), 61.5 (2-$OCH_3$), 61.1 (1-$OCH_3$), 56.3 (10-$OCH_3$), 57.6 (C-7), 30.7 (C-6), 29.7 (C-5), 27.9 (-NCOOC($\underline{C}$$H_3)_3$), 26.1 (-NHCO$\underline{C}$$H_3$). EI-MS *m/z* (%): 533 (M^+, 2), 535 ($[M+2]^+$), 433 (9), 435 (4), 83 (100). HR-EI-MS: calcd for $C_{27}H_{32}NO_8{}^{35}Cl$, 533.1853; found, 533.1816.

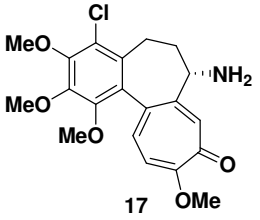
Synthesis of *N*-Boc-4-chlorodeacetylcolchicine (23).



According to the synthetic procedure from **19** to **22** described above, **23** (105.6 mg, 89%) was obtained as yellow amorphous from **20** (130 mg, 0.24 mmol).

***N*-Boc-4-chlorodeacetylcolchicine (23)**: $[\alpha]_D^{20}$: -103.2 ^{o} (*c* = 0.06, MeOH). UV (MeOH) λ_{max} (log ε): 340 (4.16), 288 (3.58), 234 (4.47), 212 (4.36) nm. CD (MeOH, 24 ^{o}C, *c* = 0.25 mM) Δε (nm): +0.74 (378), 0 (370), -7.85 (336), -4.58 (288), -14.58 (264), 0 (251), +16.93 (230), +4.32 (214), +12.68 (202). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.50 (1H, s, H-8), 7.21 (1H, d, *J* = 10.7 Hz, H-12), 6.79 (1H, d, *J* = 11.0 Hz, H-11), 4.32 (1H, ddd, *J* = 12.4, 6.4, 6.4 Hz, H-7), 4.00 (3H, s, 10-OCH_3), 3.98 (3H, s, 3-OCH_3), 3.97 (3H, s, 2-OCH_3), 3.62 (3H, s, 1-OCH_3), 3.23 (1H, dd, *J* = 13.3, 4.3 Hz, H-5β), 2.25 (1H, dddd, *J* = 12.1, 12.1, 6.0, 6.0 Hz, H-6β), 2.16 (1H, ddd, *J* = 12.9, 12.9, 5.9 Hz, H-5α), 1.62 (1H, overlapped, H-6α), 1.38 (9H, s, -C(C$\underline{H_3}$)$_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* 179.5 (C-9), 164.3 (-N$\underline{C}$OOC$(CH_3)_3$), 154.3 (C-10), 150.5 (C-7a), 150.0 (C-4), 149.8 (C-1), 146.7 (C-2), 135.1 (C-3), 134.9 (C-12), 131.7 (C-12a), 131.1 (C-8), 129.9 (C-12b), 122.0 (C-4a), 111.6 (C-11), 80.0 (-NCOO$\underline{C}$$(CH_3)_3$), 61.5 (3-$OCH_3$), 61.5 (2-$OCH_3$), 61.1 (1-$OCH_3$), 56.3 (10-$OCH_3$), 53.0 (C-7), 35.8 (C-6), 28.3 (-NCOOC($\underline{C}$$H_3)_3$), 25.9 (C-5). EI-MS *m/z* (%): 491 (M^+, 9), 493 ($[M+2]^+$), 435 (94), 437 (35). HR-EI-MS: calcd for $C_{25}H_{30}NO_7Cl$, 491.1711; found, 491.1710.

Synthesis of 4-chlorodeacetylcolchicine (17).

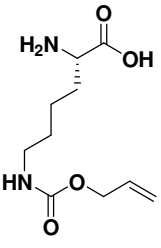


17

According to the synthetic procedure from **22** to **16** described above, **17** (385.6 mg, 99%) was obtained as yellow amorphous from **23** (485.8 mg, 0.99 mmol).

4-Chlorodeacetylcolchicine (17): $[\alpha]_D^{20}$: -31.5 ^{o} (c = 0.02, MeOH). UV (MeOH) λ_{max} (log ε): 343 (4.13), 289 (3.55), 233 (4.45), 212 (4.34) nm. CD (MeOH, 24 ^{o}C, c = 0.281 mM) Δε (nm): +0.66 (378), 0 (370), -6.49 (332), -4.22 (288), -9.01 (264), 0 (253), +25.10 (233), +5.81 (212). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, s, H-8), 7.15 (1H, d, J = 10.6 Hz, H-12), 6.79 (1H, d, J = 10.8 Hz, H-11), 4.01 (3H, s, 10-OCH_3), 3.96 (3H, s, 3-OCH_3), 3.95 (3H, s, 2-OCH_3), 3.62 (1H, overlapped, H-7), 3.61 (3H, s, 1-OCH_3), 3.19 (1H, dd, J = 13.5, 4.9 Hz, H-5β), 2.29 (1H, dddd, J = 12.6, 12.6, 4.2, 4.2 Hz, H-6β), 2.16 (1H, ddd, J = 13.4, 13.4, 6.1 Hz, H-5α), 1.55 (1H, ddd, J = 10.8, 10.8, 6.6 Hz, H-6α). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.5 (C-9), 164.0 (C-10), 153.5 (C-7a), 149.9 (C-4), 149.2 (C-1), 146.1 (C-2), 135.2 (C-3), 134.5 (C-12a), 132.6 (C-12), 131.6 (C-8), 129.7 (C-12b), 121.8 (C-4a), 111.3 (C-11), 61.3 (3-OCH_3), 61.0 (2-OCH_3), 61.0 (1-OCH_3), 53.5 (10-OCH_3), 53.5 (C-7), 38.4 (C-6), 26.5 (C-5). EI-MS m/z (%): 391 (M^+, 100), 393 ([M+2]$^+$, 35). HR-ESI-MS: calcd for $C_{20}H_{22}NO_5{}^{35}ClNa$ [M+Na]$^+$, 414.11496; found, 414.10842.

Synthesis of N^{ε}-Alloc-L-Lysine (25).

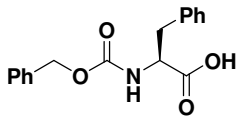


25

To a soution of L-Lysine (**24**) (100.0 mg, 0.68 mmol) in H_2O (3 mL) were added allylchloroformate (Alloc-Cl) (36 μL, 0.34 mmol) and 1N NaOH (2 drops) at 0 ^{o}C (pH 11). After stirring for 1 hour and 20 minutes at room temperature, Alloc-Cl (36 μL, 0.34 mmol) and 1N NaOH (15 drops) were added to the mixture at 0 ^{o}C again. After stirring for 6 hours and 30 minutes at room temperature, the reaction mixture was evaporated and the residue was treated with EtOH. The mixture was filtered to remove the white solid and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (20% aqueous NH_3 in MeOH / $CHCl_3$ = 20 / 80) to afford 88.9 mg (61%) of **25** as a white solid. Further, 74.9 mg (38%) of byproduct, N^{α}, N^{ε}-diAlloc- L-Lysine, was obtained as a white solid.

***N*$^{\varepsilon}$-Alloc-L-Lysine (25)**: $[\alpha]_D^{23}$: +15.1 ^{o} (c = 0.02, 1M HCl). ^{1}H-NMR (600 MHz, D_2O): δ 5.87 (1H, m, -C<u>H</u>=CH_2), 5.23 (1H, d, J = 17.3 Hz, -CH=C<u>H</u>$_2$), 5.16 (1H, d, J = 10.5 Hz, -CH=C<u>H</u>$_2$), 4.47 (2H, s, -NCOOC<u>H</u>$_2$-), 3.68 (1H, dd, J = 6.2, 6.2 Hz, C_{α}H), 3.06 (2H, m, Lys-C_{β}<u>H</u>$_2$-), 1.77 (2H, m, Lys-C_{ε}<u>H</u>$_2$-), 1.46 (2H, m, Lys-C_{γ}<u>H</u>$_2$-), 1.31 (2H, m, Lys-C_{δ}<u>H</u>$_2$-). ^{13}C-NMR (150 MHz, D_2O): δ 175.2 (-COOH), 159.2 (-NCOO-), 133.6 (-<u>C</u>H=CH_2), 118.0 (-CH=<u>C</u>H$_2$), 66.4 (-NCOO<u>C</u>H$_2$-), 55.3 (Lys-C_{α}), 40.7 (Lys-C_{β}), 30.8 (Lys-C_{ε}), 29.3 (Lys-C_{γ}), 22.3 (Lys-C_{δ}). A position of the alloc group was decided by HMBC correlation.

Synthesis of N^{α}-Cbz-L-Phenylalanine (27)[15].



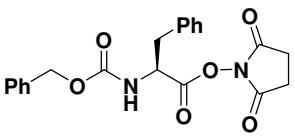
27

27 was synthesized from L-Phenylalanine (**26**) according to the known procedure in 99% yield.

***N*$^{\alpha}$-Cbz-L-Phenylalanine (27)**: $[\alpha]_D^{24}$: -34.7 ^{o} (c = 2.11, DMF). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.35-7.14 (10H, overlapped, aromatic), 5.10 (2H, s, -NCOOC<u>H</u>$_2$Ph), 4.70 (1H, ddd, J = 6.5,

6.5, 6.5 Hz, $C_\alpha\underline{H}$), 3.21 (1H, dd, *J* = 13.8, 5.4 Hz, -NC_αH-$C\underline{H}_2$-), 3.11 (1H, dd, *J* = 14.0, 6.1 Hz, -NC_αH-$C\underline{H}_2$-). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.9 (-COOH), 155.8 (-NCOO-), 136.0; 135.4; 129.8; 128.7; 128.5; 128.2; 128.1; 127.3 (aromatic), 67.2 (-NCOO$\underline{C}H_2$Ph), 54.5 (C_α), 37.7 (-NC_αH-$\underline{C}H_2$-).

Synthesis of Z-L-Phe-OSu (28)[16].

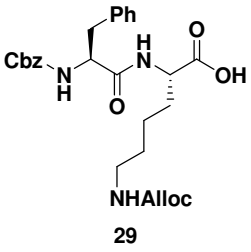


28

28 was synthesized from **27** according to the known procedure in 81% yield.

Z-L-Phe-OSu (28): $[\alpha]_D^{23}$: -55.3 ^{o} (*c* = 1.04, DMF). UV (MeOH) λ_{max} (log ε): 208 (4.13) nm. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.34-7.25 (10H, overlapped, aromatic), 5.20 (1H, br-d, *J* = 7.6 Hz, -NH), 5.13-5.01 (3H, overlapped, -NCOO$C\underline{H}_2$Ph), $C_\alpha\underline{H}$), 3.32 (1H, dd, *J* = 14.3, 5.7 Hz, -NC_αH-$C\underline{H}_2$-), 3.22 (1H, dd, *J* = 14.2, 6.1 Hz, -NC_αH-$C\underline{H}_2$-), 2.83 (4H, s, -CH_2CH_2-). ^{13}C-NMR (100MHz, $CDCl_3$): δ 168.6 (-COO-), 167.5 (-N$\underline{C}OOCH_2$Ph), 155.3 (-N$\underline{C}OCH_2$-), 135.9; 134.3; 129.6; 128.7; 128.5; 128.2; 127.5 (aromatic), 67.3 (-NCOO$\underline{C}H_2$Ph), 52.9 (C_α), 37.9 (-NC_αH-$\underline{C}H_2$-), 25.5 (-$\underline{C}H_2\underline{C}H_2$-). ESI-MS: 419 $[M+Na]^+$.

Synthesis of Z-L-Phe-L-Lys(Alloc) (29)[16].

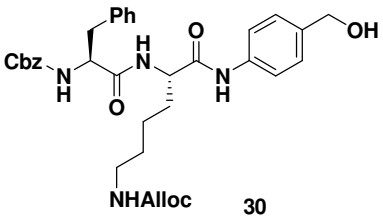


29

29 was synthesized from **28** according to the known procedure in 79% yield.

Z-L-Phe-L-Lys(Alloc) (29): $[\alpha]_D^{22}$: -15.1 ^{o} (*c* = 0.20, DMF). UV (MeOH) λ_{max} (logε): 208 (3.96) nm. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.32-7.20 (10H, overlapped, aromatic), 5.90 (1H, m, -C$\underline{H}$=CH_2), 5.29 (1H, dd, *J* = 17.2, 2.5 Hz, -CH=C$\underline{H}_2$), 5.15 (1H, dd, *J* = 10.6, 1.3 Hz, -CH=C$\underline{H}_2$), 5.05 (1H, d, *J* = 12.6 Hz, -NCOOC$\underline{H}_2$Ph), 4.99 (1H, d, *J* = 12.6 Hz, -NCOOC$\underline{H}_2$Ph), 4.51 (2H, dd, *J* = 5.5, 1.1 Hz, -NCOOC$\underline{H}_2$CH=CH_2), 4.29 (1H, dd, *J* = 7.5, 7.5 Hz, Phe-C_αH), 4.08 (1H, m, Lys-C_αH), 3.18-3.01 (3H, overlapped, -NC_αH-$C\underline{H}_2$-, Lys-$C_\beta\underline{H}_2$-), 2.86 (1H, dd, *J* = 13.4, 8.4 Hz, -C_αHC$\underline{H}_2$Ph), 1.78 (1H, m, Lys-$C_\varepsilon H_2$-), 1.62 (1H, m, Lys-$C_\varepsilon H_2$-), 1.39 (2H, m, Lys-$C_\gamma H_2$-), 1.29 (2H, m, Lys-$C_\delta H_2$-). ^{13}C-NMR (100 MHz, *d*$_6$-DMSO): δ 174.1 (-COOH), 171.2 (-N$\underline{C}OC_\alpha$H-), 156.0 (-N$\underline{C}OOCH_2CH$=CH_2), 155.8 (-N$\underline{C}OOCH_2$Ph), 138.1; 137.1 (aromatic), 133.6 (-$\underline{C}$H=CH_2), 129.2; 128.3; 128.1; 127.7; 127.5; 126.3 (aromatic), 117.0 (-CH=$\underline{C}H_2$), 65.2 (-NCOO$\underline{C}H_2$Ph), 64.4 (-NCOO$\underline{C}H_2$CH=CH_2), 56.3 (Phe-C_α), 53.8 (Lys-C_α), 38.3 (-NC_αH-$\underline{C}H_2$Ph), 37.8 (Lys-C_β), 30.4 (Lys-C_ε), 28.6 (Lys-C_γ), 23.0 (Lys-C_δ). ESI-MS: 534 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{27}H_{33}N_3O_7Na$ $[M+Na]^+$, 534.22162; found, 534.21757.

Synthesis of Z-L-Phe-L-Lys(Alloc)-PABOH (30).



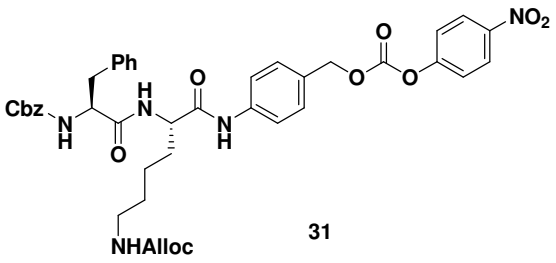
30

To the solution of **29** (125.1 mg, 0.245mmol), HOBt (41.4 mg, 0.270 mmol) and *p*-aminobenzylalcohol (60.3 mg, 0.490 mmol) in THF (3.0 mL) were added NMM (28.3 μL, 0.257 mmol) and EDCI (49.3 mg, 0.257 mmol) at 0 ^{o}C under argon atmosphere. The reaction mixture was stirred for 50 minutes at 0 ^{o}C and 2 hours and 20 minutes at room temperature. Then the reaction mixture was acidified to pH 3 with a saturated aqueous citric acid and the whole mixture was extracted three times with AcOEt. The combined organic layers were washed with

H_2O and dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was treated with Et_2O and the mixture was allowed to stand at 0 °C. The precipitate was filtered and washed with cold Et_2O to afford 144.9 mg (96%) of **30** as yellow solid.

Z-L-Phe-L-Lys(Alloc)-PABOH (30): $[\alpha]_D^{23}$: -7.1 ° (*c* = 0.08, DMF). UV (MeOH) λ_{max} (log ε): 248 (4.22), 224 (3.81) nm. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ (3 drops)): δ 7.52 (2H, d, *J* = 8.4 Hz, aromatic), 7.36-7.15 (12H, overlapped, aromatic), 5.90 (1H, m, -C<u>H</u>=CH_2), 5.30 (1H, d, *J* = 17.0 Hz, -CH=C<u>H</u>$_2$), 5.21 (1H, d, *J* = 10.3 Hz, -CH=C<u>H</u>$_2$), 5.07 (1H, d, *J* = 12.5 Hz, NCOOC<u>H</u>$_2$Ph), 5.02 (1H, d, *J* = 12.5 Hz, NCOOC<u>H</u>$_2$Ph), 4.58 (2H, s, -C<u>H</u>$_2$OH), 4.55 (2H, d, *J* = 5.3 Hz, -NCOOC<u>H</u>$_2$CH=CH_2), 4.31 (1H, dd, *J* = 7.1, 7.1 Hz, Phe-C_αH), 4.20 (1H, br-dd, *J* = 6.7, 6.7 Hz, Lys-C_αH), 3.12 (2H, m, Lys-C_β<u>H</u>$_2$-), 3.03 (1H, dd, *J* = 13.6, 7.0 Hz, -NC_αH-C<u>H</u>$_2$Ph), 2.93 (1H, dd, *J* = 13.6, 7.5 Hz, -NC_αH-C<u>H</u>$_2$Ph), 1.79 (1H, ddd, *J* = 13.9, 13.9, 7.3 Hz, Lys-C_ε<u>H</u>$_2$-), 1.65 (1H, ddd, *J* = 14.1, 14.1, 8.7 Hz, Lys-C_ε<u>H</u>$_2$-), 1.40 (2H, m, Lys-C_γ<u>H</u>$_2$-), 1.30 (2H, m, Lys-C_δ<u>H</u>$_2$-). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ (3 drops)): δ 171.6 (-<u>C</u>ONH- (Lys)), 170.8 (-<u>C</u>ONH- (Phe)), 156.5 (-N<u>C</u>OOCH$_2$CH=CH_2), 156.2 (-N<u>C</u>OOCH$_2$Ph), 137.1; 136.8; 136.3; 135.9 (aromatic), 133.5 (-<u>C</u>H=CH_2), 132.3; 129.0; 128.3; 128.0; 127.7; 127.4; 126.7; 120.0 (aromatic), 117.6 (-CH=<u>C</u>H$_2$), 66.8 (-NCOO<u>C</u>H$_2$Ph), 65.8 (-<u>C</u>H$_2$OH), 64.0 (-NCOO<u>C</u>H$_2$CH=CH_2), 56.1 (Phe-C_α), 55.0 (Lys-C_α), 38.5 (-C_αH-<u>C</u>H$_2$Ph), 31.8 (Lys-C_β), 28.3 (Lys-C_ε), 27.9 (Lys-C_γ), 22.3 (Lys-C_δ). ESI-MS: 639 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{34}H_{40}N_4O_7Na$ $[M+Na]^+$, 639.27947; found, 639.26957.

Synthesis of Z-L-Phe-L-Lys(Alloc)-PABC-PNP (31).



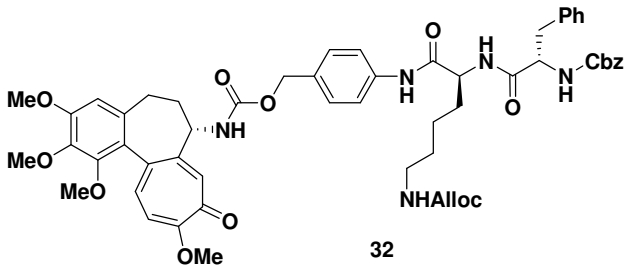
31

To a stirred solution of **30** (206.8 mg, 0.34mmol) in THF (16 mL) were added pyridine (69 μL, 0.85 mmol) and *p*-nitrophenylchloroformate (137.1 mg, 0.68 mmol) under argon atmosphere at 0 °C. After stirring for 2 hours at room temperature, a saturated aqueous citric acid was added to the reaction mixture and the whole mixture was extracted three times with AcOEt. The combined organic layers were washed with H_2O and dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 1 / 99) to afford 251.3 mg (95%) of **31** as white solid.

Z-L-Phe-L-Lys(Alloc)-PABC-PNP (31): $[\alpha]_D^{23}$: -4.7 ° (*c* = 0.15, DMF). UV (MeOH) λ_{max} (log ε): 253 (4.45), 226 (4.11), 210 (4.65) nm. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ (3 drops)): δ 8.27 (2H, d, *J* = 9.0 Hz, aromatic), 7.63 (2H, d, *J* = 8.4 Hz, aromatic), 7.40-7.16 (14H, overlapped, aromatic), 5.90 (1H, m, -C<u>H</u>=CH_2), 5.30 (1H, d, *J* = 17.0 Hz, -CH=C<u>H</u>$_2$), 5.24 (2H, s, -C<u>H</u>$_2$OCOO-), 5.22 (1H, d, *J* = 10.3 Hz, -CH=C<u>H</u>$_2$), 5.07 (2H, m, -NCOOC<u>H</u>$_2$Ph), 4.56 (2H, br-d, *J* = 5.1 Hz, -NCOOC<u>H</u>$_2$CH=CH_2), 4.31 (1H, dd, *J* = 7.1, 7.1 Hz, Phe-C_αH), 4.20 (1H, dd, *J* = 6.9, 6.9 Hz, Lys-C_αH), 3.14 (2H, m, Lys-C_β<u>H</u>$_2$-), 3.00 (2H, m, -C_αH-C<u>H</u>$_2$Ph), 1.79 (1H, m, Lys-C_ε<u>H</u>$_2$-), 1.64 (1H, m, Lys-C_ε<u>H</u>$_2$-), 1.40 (2H, m, Lys-C_γ<u>H</u>$_2$-), 1.25 (2H, m, Lys-C_δ<u>H</u>$_2$-). ^{13}C-NMR (100 MHz, d_6-DMSO): δ 171.4 (-<u>C</u>ONH- (Lys)), 171.2 (-<u>C</u>ONH- (Phe)), 156.0 (-N<u>C</u>OOCH$_2$CH=CH_2), 155.8 (-N<u>C</u>OOCH$_2$Ph), 155.3 (-CH_2O<u>C</u>OO-), 152.0; 145.2; 139.4; 138.1; 137.0 (aromatic), 133.6 (-<u>C</u>H=CH_2), 129.4; 129.3; 129.2; 128.3; 128.0; 127.7; 127.5; 126.2; 125.4; 122.6; 119.2 (aromatic), 117.0 (-CH=<u>C</u>H$_2$), 70.3 (-NCOO<u>C</u>H$_2$Ph), 65.2 (-<u>C</u>H$_2$OCOO-), 64.5 (-NCOO<u>C</u>H$_2$CH=CH_2), 56.3 (Phe-C_α), 55.4 (Lys-C_α), 38.3 (-C_αH-<u>C</u>H$_2$Ph), 37.8 (Lys-C_β), 31.4 (Lys-C_ε), 28.6 (Lys-C_γ), 22.9

(Lys-C_δ). ESI-MS: 804 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{41}H_{43}N_5O_{11}Na$ $[M+Na]^+$, 804.28568 found, 804.28531.

Synthesis of Z-L-Phe-L-Lys(Alloc)-PABC-deacetylcolchicine (32).

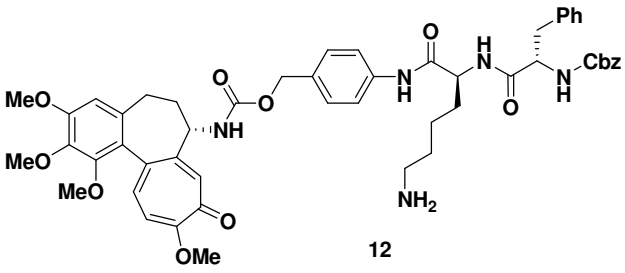


32

To a stirred solution of **31** (1.15 g, 1.47 mmol) in NMP (30 mL) were added Et_3N (0.23 mL, 1.62 mmol) and **15** (578.9 mg, 1.62 mmol) under argon atmosphere at 0 °C. After stirring for 37 hours at room temperature, the reaction mixture was diluted with AcOEt. The whole mixture was washed three times with H_2O and washed three times with saturated aqueous $NaHCO_3$. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (MeOH / $CHCl_3$ = 2 / 98) to afford 1.18 g (80%) of **32** as yellow amorphous.

Z-L-Phe-L-Lys(Alloc)-PABC-deacetylcolchicine (32): $[\alpha]_D^{24}$: -83.3 ° (*c* = 0.19 MeOH). UV (MeOH) λ_{max} (log ε): 349 (4.16), 292 (3.53), 246 (4.61), 223 (4.42) nm. CD (MeOH, 24 °C, *c* = 0.14 mM) Δε (nm): -0.20 (397), -8.37 (344), -3.64 (307), -6.96 (270), 0 (252), +15.34 (232), 0 (215), -2.79 (211), 0 (208). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, s, H-8), 7.49 (2H, br-d, *J* = 8.3 Hz, aromatic), 7.33-7.15 (13H, overlapped, H-12, aromatic), 6.82 (1H, d, *J* = 10.7 Hz, H-11), 6.53 (1H, s, H-4), 6.02 (1H, br-s, -NH), 5.86 (1H, m, -C<u>H</u>=CH_2), 5.74 (1h, br-s, -NH), 5.26 (1H, d, *J* = 17.3 Hz, -CH=C<u>H_2</u>), 5.16 (1H, d, *J* = 10.5 Hz, -CH=C<u>H_2</u>), 5.06 (1H, d, *J* = 12.9 Hz, NCOOC<u>H_2</u>Ph), 4.99 (1H, d, *J* = 12.9 Hz, NCOOC<u>H_2</u>Ph), 4.93 (1H, d, *J* = 12.2 Hz, -C_7H-NCOOC<u>H_2</u>-), 4.86 (1H, d, *J* = 12.0 Hz, -C_7H-NCOOC<u>H_2</u>-), 4.53 (2H, br-d, *J* = 3.7 Hz, -NCOOC<u>H_2</u>CH=CH_2), 4.47-4.41 (2H, overlapped, Phe-C_αH, H–7), 4.28 (1H, m, Lys-C_αH), 3.94 (6H, s, 3-OCH_3, 2-OCH_3), 3.90 (3H, s, 10-OCH_3), 3.64 (3H, s, 1-OCH_3), 3.15 (1H, m, Lys-C_β<u>H_2</u>-), 3.08-3.00 (3H, overlapped, Lys-C_β<u>H_2</u>-, -C_αH-C<u>H_2</u>Ph), 2.50 (1H, dd, *J* = 12.9, 6.3 Hz, H-5β), 2.38 (1H, ddd, *J* = 12.9, 12.9, 6.3 Hz, H-5α), 2.24 (1H, dddd, *J* = 12.4, 12.4, 6.2, 6.2 Hz, H-6β), 1.81 (2H, m, Lys-C_ε<u>H_2</u>-), 1.67 (1H, m, H-6α), 1.37 (2H, m, Lys-C_γ<u>H_2</u>-), 1.26 (2H, m, Lys-C_δ<u>H_2</u>-). ^{13}C-NMR (100 MHz, *d_6*-DMSO): δ 179.5 (C-9), 171.4 (-<u>C</u>ONH- (Lys)), 170.6 (-<u>C</u>ONH- (Phe)), 164.0 (C-10), 156.5 (-N<u>C</u>OOCH_2CH=CH_2), 155.3 (-N<u>C</u>OOCH_2Ph), 153.5 (-C_7H-N<u>C</u>OOCH_2-), 151.4 (C-7a), 151.1 (C-4), 141.6 (C-1), 137.5 (C-2), 136.6 (C-3), 136.1; 135.2 (aromatic), 134.2 (C-12a), 132.5 (C-12), 132.0 (-<u>C</u>H=CH_2, 131.2 (aromatic), 129.3 (C-8), 128.8; 128.6; 128.5; 128.4 (aromatic) 128.1 (C-12b), 127.9; 126.9; 125.5 (aromatic), 120.0 (C-4a), 118.0 (aromatic), 112.4 (-CH=<u>C</u>H_2), 107.3 (C-11), 67.0 (-NCOO<u>C</u>H_2Ph), 66.4 (-C_7H-NCOO<u>C</u>H_2-), 66.0 (-NCOO<u>C</u>H_2CH=CH_2), 61.4 (1-OCH_3, 3-OCH_3), 61.3 (2-OCH_3), 56.4 (Phe-C_α), 56.2 (10-OCH_3), 56.1 (Lys-C_α), 55.4 (C-7), 53.8 (-C_αH-<u>C</u>H_2Ph), 38.7 (Lys-C_β), 37.0 (C-6), 31.5 (Lys-C_ε), 29.9 (Lys-C_γ), 28.5 (Lys-C_δ), 22.3 (C-5). ESI-MS: 1022 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{55}H_{61}N_5O_{13}Na$ $[M+Na]^+$, 1022.4158; found, 1022.4156.

Synthesis of Z- L-Phe-L-Lys-PABC-deacetylcolchicine (12).

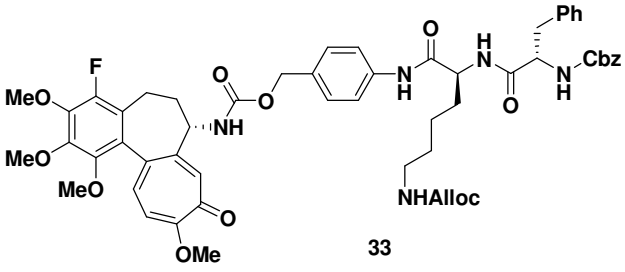


12

To a stirred solution of **32** (54.8 mg, 0.055 mmol) and $Pd(PPh_3)_4$ (6.4 mg, 0.0055 mmol) in degassed THF (0.5 mL) was added degassed morpholine (0.1 mL) under argon atmosphere at 0 °C. After stirring for 1 hour and 30 minutes at room temperature, the reaction mixture was treated with Et_2O and the mixture was filtered to remove the yellow solid. The filtrate was evaporated under reduced pressure and the residure was purified by amino-silica gel open column chromatography (MeOH / $CHCl_3$ = 1 / 99) to afford 29.6 mg (59%) of **12** as yellow amorphous.

Z- L-Phe-L-Lys-PABC-deacetylcolchicine (12): $[\alpha]_D^{23}$: -75.2 ° (*c* = 0.09, MeOH). UV (MeOH) λ_{max} (log ε): 343 (4.14), 290 (3.60), 246 (4.57), 223 (4.42) nm. CD (MeOH, 24 °C, *c* = 0.19 mM) Δε (nm): +0.54 (378), 0 (374), -8.07 (340), -4.46 (288), -11.07 (264), 0 (246), +22.19 (230), +4.89 (209), +13.00 (201). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.55 (2H, br-d, *J* = 8.4 Hz, aromatic), 7.51 (1H, s, H-8), 7.35-7.16 (13H, overlapped, H-12, aromatic), 6.81 (1H, d, *J* = 10.8 Hz, H-11), 6.53 (1H, s, H-4), 6.10 (1H, br-s, -NH), 5.58 (2H, br-s, -NH), 5.08 (1H, d, *J* = 12.6, -NCOOC$\underline{H}_2$Ph), 5.06 (1H, d, *J* = 12.4, -NCOOC$\underline{H}_2$Ph), 4.99 (1H, d, *J* = 12.1 Hz, -C_7H-NCOOC$\underline{H}_2$-), 4.88 (1H, d, *J* = 12.1 Hz, -C_7H-NCOOC$\underline{H}_2$-), 4.44 (1H, ddd, *J* = 12.2, 6.2, 6.2 Hz, H-7), 4.38 (1H, dd, *J* = 14.5, 7.0 Hz, Phe-C_αH), 3.97 (3H, s, 3-OCH_3), 3.94 (3H, s, 2-OCH_3), 3.90 (3H, s, 10-OCH_3), 3.64 (3H, s, 1-OCH_3), 3.37 (1H, dd, *J* = 8.2, 4.3 Hz, Lys-C_αH), 3.21-3.14 (3H, overlapped, Lys-$C_\beta\underline{H}_2$-, -C_αHC$\underline{H}_2$Ph), 3.03 (1H, dd, *J* = 13.7, 7.7 Hz, -C_αHC$\underline{H}_2$Ph), 2.51 (1H, dd, *J* = 14.3, 6.2 Hz, H-5β), 2.39 (1H, ddd, *J* = 13.1, 13.1, 7.0 Hz, H-5α), 2.27 (1H, dddd, *J* = 12.4, 12.4, 6.2, 6.2, H-6β), 1.85 (1H, m, Lys-$C_\varepsilon\underline{H}_2$), 1.75 (1H, m, Lys-$C_\varepsilon\underline{H}_2$), 1.53 (1H, ddd, J = 14.0, 7.9, 7.9, H-6α), 1.39 (2H, m, Lys-$C_\gamma\underline{H}_2$-), 1.30 (2H, m, Lys-$C_\delta\underline{H}_2$-). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.4 (C-9), 173.3 (-C$\underline{O}$NH- (Lys)), 171.0 (-C$\underline{O}$NH- (Phe)), 164.0 (C-10), 155.3 (-N$\underline{C}$OOCH_2Ph), 153.4 (-C_7H-N$\underline{C}$OOCH_2-), 151.1 (C-7a), 141.5 (C-4), 137.7 (C-1), 136.6 (C-2), 136.2 (C-3), 136.0; 135.1 (aromatic), 134.2 (C-12a), 131.7 (C-12), 131.1 (aromatic), 129.3 (C-8), 128.9; 128.53; 128.46 (aromatic), 128.1 (C-12b), 127.9; 126.9; 125.5 (aromatic), 119.4 (C-4a), 112.2 (aromatic), 107.3 (C-11), 66.9 (-NCOO$\underline{C}H_2$Ph), 66.5 (-C_7H-NCOO$\underline{C}H_2$-), 61.3 (1-OCH_3, 3-OCH_3,), 56.4 (2-OCH_3), 56.2 (Phe-C_α), 56.0 (10-OCH_3), 55.1 (Lys-C_α), 53.8 (C-7), 38.9 (-C_αH-$\underline{C}H_2$Ph), 38.7 (Lys-C_β), 37.0 (C-6), 34.1 (Lys-C_ε), 29.6 (Lys-C_γ), 28.7 (Lys-C_δ), 22.7 (C-5). ESI-MS: 938 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{51}H_{57}N_5O_{11}Na$ $[M+Na]^+$, 938.3947; found, 938.3942.

Synthesis of Z- L-Phe-L-Lys(Alloc)-PABC-4-fluorodeacetylcolchicine (33).



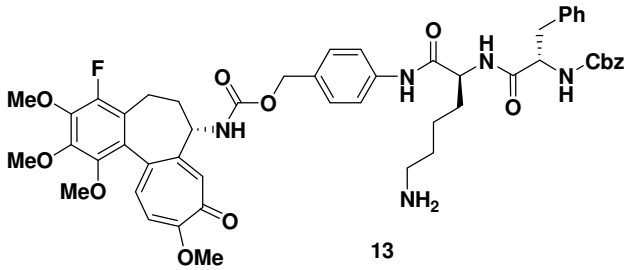
33

According to the synthetic procedure from **15** and **31** to **32** described above, **33** (85.5 mg, quantitative yield) was obtained as yellow amorphous from **16** (31.3 mg, 0.083 mmol) and **31** (69 mg, 0.091 mmol).

Z- L-Phe-L-Lys(Alloc)-PABC-4-fluorodeacetylcolchicine (33): $[\alpha]_D^{23}$: -79.3 ° (*c* = 0.18, MeOH). UV

(MeOH) λ_{max} (log ε): 345 (4.17), 291 (3.56), 247 (4.60), 223 (4.44) nm. CD (MeOH, 24 °C, *c* = 0.14 mM) Δε (nm): +0.79 (378), 0 (373), -8.68 (336), -4.84 (289), -12.48 (263), 0 (245), +22.72 (230), +1.20 (211). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.55 (1H, s, H-8), 7.48 (2H, d, *J* = 8.1 Hz, aromatic), 7.31-7.14 (13H, overlapped, H-12, aromatic), 6.80 (1H, d, *J* = 11.0 Hz, H-11), 6.50 (1H, br-s, -NH), 5.99 (1H, br-s, -NH), 5.86 (1H, m, -C<u>H</u>=CH_2), 5.74 (1H, br-s, -NH), 5.26 (1H, d, *J* = 17.2 Hz, -CH=C<u>H</u>$_2$), 5.16 (1H, d, *J* = 10.2 Hz, -CH=C<u>H</u>$_2$), 5.06 (1H, d, *J* = 12.4 Hz, -NCOOC<u>H</u>$_2$Ph), 4.97 (1H, d, *J* = 12.7 Hz, -NCOOC<u>H</u>$_2$Ph), 4.94 (1H, d, *J* = 12.4 Hz, -C_7H-NCOOC<u>H</u>$_2$-), 4.87 (1H, d, *J* = 12.1 Hz, -C_7H-NCOOC<u>H</u>$_2$-), 4.53 (2H, br-d, *J* = 4.8 Hz, -NCOOC<u>H</u>$_2$CH=CH_2), 4.44-4.37 (2H, overlapped, Phe-C_αH, H-7), 4.26 (1H, m, Lys-C_αH), 4.00 (3H, s, 3-OCH_3), 3.99 (3H, s, 2-OCH_3), 3.94 (3H, s, 10-OCH_3), 3.60 (3H, s, 1-OCH_3), 3.16 (1H, m, Lys-C_β<u>H</u>$_2$-), 3.08-2.97 (4H, overlapped, H-5β, Lys-C_β<u>H</u>$_2$-, -C_αHC<u>H</u>$_2$Ph), 2.21 (1H, dddd, *J* = 12.3, 12.3, 6.1, 6.1 Hz, H-6β), 2.02 (1H, m, H-5α), 1.79 (2H, m, H-6α, Lys-C_ε<u>H</u>$_2$-), 1.67 (1H, m, Lys-C_ε<u>H</u>$_2$-), 1.36 (2H, m, Lys-C_γ<u>H</u>$_2$-), 1.28 (2H, m, Lys-C_δ<u>H</u>$_2$-). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 179.4 (C-9), 171.4 (-<u>C</u>ONH- (Lys)), 170.6 (-<u>C</u>ONH- (Phe)), 164.3 (C-10), 156.5 (-N<u>C</u>OOCH_2CH=CH_2), 156.2 (-N<u>C</u>OOCH_2Ph), 155.4 (-C_7H-N<u>C</u>OOCH_2-), 151.0 (C-7a), 149.0 (d, *J* = 242.4 Hz, C-4), 146.9 (d, *J* = 2.3 Hz, C-1), 146.1 (d, *J* = 4.6 Hz, C-2), 141.8 (d, *J* = 13.8 Hz, C-3), 137.8; 136.6; 136.1 (aromatic), 135.5 (C-12a), 134.8 (C-12), 132.5 (<u>C</u>H=CH_2), 131.9 (aromatic), 131.2 (C-8), 129.2; 128.7; 128.5; 128.4 (aromatic), 128.3 (d, *J* = 4.6 Hz, C-12b), 128.1; 127.9; 126.9 (aromatic), 120.6 (d, *J* = 16.0 Hz, C-4a), 120.0 (aromatic), 118.0 (CH=<u>C</u>H$_2$), 112.1 (C-11), 67.0 (-NCOO<u>C</u>H$_2$Ph), 66.5 (-C_7H-NCOO<u>C</u>H$_2$-), 66.0 (-NCOO<u>C</u>H$_2$CH=CH_2), 61.7 (1-OCH_3, 3-OCH_3), 61.5 (2-OCH_3), 56.4 (Phe-C_α), 56.3 (10-OCH_3), 55.4 (Lys-C_α), 53.7 (C-7), 38.8 (-C_αH<u>C</u>H$_2$Ph), 38.6 (Lys-C_β), 35.8 (C-6), 31.5 (Lys-C_ε), 28.5 (Lys-C_γ), 22.3 (Lys-C_δ), 20.7 (C-5). ESI-MS: 1040 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{55}H_{60}N_5O_{13}FNa$ $[M+Na]^+$, 1040.40693; found, 1040.40101.

Synthesis of Z-L-Phe-L-Lys-PABC-4-fluorodeacetylcolchicine (13).



13

According to the synthetic procedure from **32** to **12** described above, **13** (15.5 mg, 55%) was obtained as yellow amorphous from **33** (30.3 mg, 0.030 mmol).

Z-L-Phe-L-Lys-PABC-4-fluorodeacetylcolchicine (13): $[\alpha]_D^{23}$: -70.8 ° (*c* = 0.11, MeOH). UV (MeOH) λ_{max} (log ε): 345 (4.15), 291 (3.60), 247 (4.58), 223 (4.44) nm. CD (MeOH, 24 °C, *c* = 0.09 mM) Δε (nm): +0.54 (377), 0 (374), -7.95 (343), -4.64 (297), -10.68 (263), 0 (247), +23.09 (231), +3.24 (211). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 (2H, d, *J* = 8.1 Hz, aromatic), 7.56 (1H, s, H-8), 7.36-7.15 (13H, overlapped, H-12, aromatic), 6.80 (1H, d, *J* = 10.8 Hz, H-11), 6.14 (1H, br-s, -NH), 5.70 (1H, br-s, -NH), 5.60 (1H, br-s, -NH), 5.08 (1H, d, *J* = 12.3 Hz, -NCOOC<u>H</u>$_2$Ph), 5.04 (1H, d, *J* = 12.1 Hz, -NCOOC<u>H</u>$_2$Ph), 4.99 (1H, d, *J* = 12.1 Hz, -C_7H-NCOOC<u>H</u>$_2$-), 4.89 (1H, d, *J* = 12.1 Hz, -C_7H-NCOOC<u>H</u>$_2$-), 4.44-4.37 (2H, overlapped, Phe-C_αH, H-7), 4.01 (3H, s, 3-OCH_3), 3.99 (3H, s, 2-OCH_3), 3.97 (3H, s, 10-OCH_3), 3.60 (3H, s, 1-OCH_3), 3.37 (1H, dd, *J* = 8.1, 4.2 Hz, Lys-C_αH), 3.17 (1H, m, Lys-C_β<u>H</u>$_2$-), 3.10-2.99 (4H, overlapped, H-5β, Lys-C_β<u>H</u>$_2$-, -C_αHC<u>H</u>$_2$Ph), 2.23 (1H, dddd, *J* = 12.5, 12.5, 6.2, 6.2 Hz, H-6β), 2.01 (1H,

ddd, *J* = 12.9, 12.9, 5.3 Hz, H-5α), 1.85 (1H, ddd, *J* = 11.7, 11.7, 6.8 Hz, H-6α), 1.75 (1H, ddd, *J* = 11.7, 11.7, 6.8 Hz, Lys-$C_\varepsilon\underline{H_2}$-), 1.52 (1H, ddd, *J* = 14.2, 14.2, 7.9 Hz, Lys-$C_\varepsilon\underline{H_2}$-), 1.38 (2H, m, Lys-$C_\gamma\underline{H_2}$-), 1.29 (2H, m, Lys-$C_\delta\underline{H_2}$-). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 179.4 (C-9), 173.2 (-$\underline{C}$ONH- (Lys)), 170.8 (-$\underline{C}$ONH- (Phe)), 164.4 (C-10), 155.9 (-N$\underline{C}$OOCH_2Ph), 155.2 (-C_7H-N$\underline{C}$OOCH_2-), 150.3 (C-7a), 149.0 (d, *J* = 242.4 Hz, C-4), 146.9 (d, *J* = 3.2 Hz, C-1), 146.1 (d, *J* = 4.6 Hz, C-2), 141.8 (d, *J* = 4.2 Hz, C-3), 137.8; 136.6; 136.2 (aromatic), 135.4 (C-12a), 134.5 (C-12), 131.6 (aromatic), 131.2 (C-8), 129.3; 129.0; 128.6; 128.5 (aromatic), 128.3 (d, *J* = 3.7 Hz, C-12b), 128.2; 128.0; 127.0 (aromatic), 120.6 (d, *J* = 16.0 Hz, C-4a), 119.4 (aromatic), 111.8 (C-11), 67.0 (-NCOO$\underline{C}H_2$Ph), 66.7 (-C_7H-NCOO$\underline{C}H_2$-), 61.7 (1-OCH_3, 3-OCH_3), 61.5 (2-OCH_3), 56.5 (Phe-C_α), 56.3 (10-OCH_3), 55.1 (Lys-C_α), 53.7 (C-7), 38.9 (-$C_\alpha H\underline{C}H_2$Ph), 38.7 (Lys-$C_\beta$), 36.0 (C-6), 34.0 (Lys-C_ε), 28.8 (Lys-C_γ), 22.7 (Lys-C_δ), 20.7 (d, *J* = 1.8 Hz, C-5). ESI-MS: 956 $[M+Na]^+$. HR-ESI-MS: calcd for $C_{51}H_{56}N_5O_{11}FNa$ $[M+Na]^+$, 956.38580; found, 956.38966.

1. ***In vitro***

Cell Culture. Human lung and colorectal cancer cell lines, A549 and HT29, respectively, were obtained from ATCC. A549 and HT29 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) (D6046, D6046) and D-MEM/F-12 medium (D8062, Sigma) with 10% heat-inactivitated fetal bovine serum (FBS) and 5 mg/mL gentamicin, respectively, at 37 ^oC in a humidified atmosphere containing 5% CO_2.

Growth Inhibition Assay. A 190 μL volume of an exponentially growing cell suspension (1 x 10^4 cells/1.9 mL) was seeded into a 96-well microtiter plate, and 10 μL of each drug at various concentrations was added 24 h after seeding of the tumor cells. After incubation for 96 h at 37 ^oC, 10 μL of Tetracolor ONE (Seikagaku Biobusiness Corporation, Tokyo, Japan) was added to each well, and the plates were incubated for a further 1 h at 37 ^oC. After incubation, optical density was measured at 450 nm with a microplate reader (SpectraMaxPlus, Molecular Devices, CA), and the concentration causing 50% inhibition of cell proliferation (IC_{50}) was calculated by linear regression analysis of the liner portion of the growth curves.

2. ***In vivo***

Animals. Inbred specific pathogen-free 5-6-week-old male BALB/c nude mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were kept in plastic cages, given a standard diet (MF, Oriental Yeast Industry Co., Tokyo, Japan) and were allowed free access to water. The temperature and humidity were kept at 24±1 and 55±10%, respectively. *In vivo* antitumor experiment was performed according to our internal and ethics committee regulations.

Cells and culture. The human colorectal cancer cell line (HCT116) was purchased from American Type Culture Collection (ATCC, USA) and was maintained in Dulbecco's modified eagle's medium （Sigma, SL, USA）containing 10% heat-inactivated fetal bovine serum (FBS) The cells were subcultured serially *in vitro* and adjusted to the appropriate concentrations before use.

Antitumor experiments. After transplanting HCT116 cells (2 x 10^6 cells/mouse) subcutaneously to the inguinal region of nude mice, the mice were grouped (5 mice/group) on the day when the estimated tumor volume calculated by the following formula (A) reached about 200 mm^3 (Day 0). Derivatives were administered intraperitoneally 10 times on days 1-5 & 8-12. Colchicine was administered intraperitoneally 3 time on days 1, 3 and 5 as positive control. Physiologic saline was administered as negative control with the same administration schedule of derivatives. Tumors were excised on day 21, and tumor growth inhibition rate (IR (%), formula (B)) was calculated from the tumor weights.

Estimated tumor volume = 1/2 × long diameter × short diameter × short diameter (A)

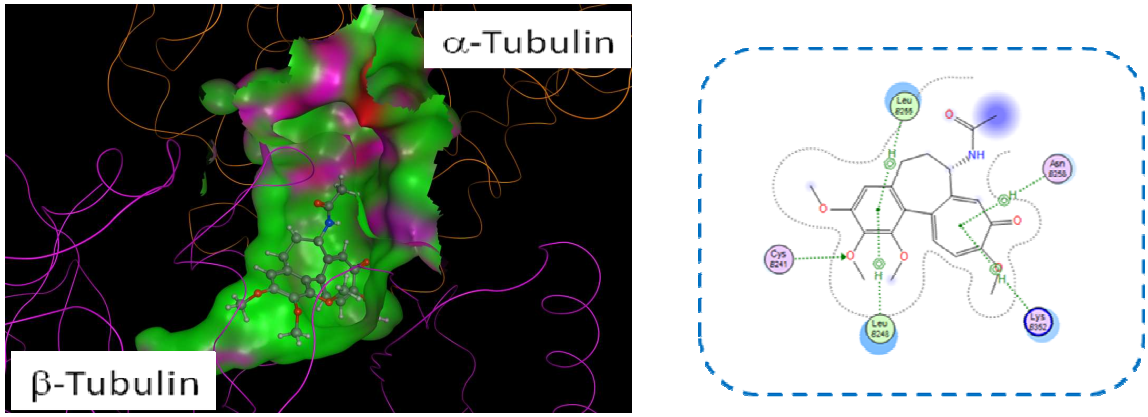
IR% = (1 – mean tumor weight in the tested group/mean tumor weight in the control group) × 100 (B)

Drug release. We chose bovine spleen cathepsin B (EC 3.4.22.1, *Mw* ca. 40000) and prepared stock solution by dissolving the lyophilized solid. For each assay, 1 mM DMSO solution of pro-drug compound (4 μM) was added to the enzyme solution (96 μL) and the concentration of substrate was adjusted to 0.04 mM. The mixture was stirred with vortex, stood at 37 °C and monitored the reaction course using HPLC analysis. The mixture of 20 μL was removed at various time points and quenched by MeOH of 20 μL. The reaction mixture quenched was stored at 0 °C and 10 μL of the mixture was injected to the HPLC analysis. (HPLC condition: Inertsil ODS-2 4.6 x 250 mm, solvent A: 0.1 % (v/v) H_3PO_4, solvent B: CH_3CN; 0 min B conc. 15%; 30 min B conc. 100%, column oven 40 °C, flow rate 0.5 mL/min, λ = 254 nm).

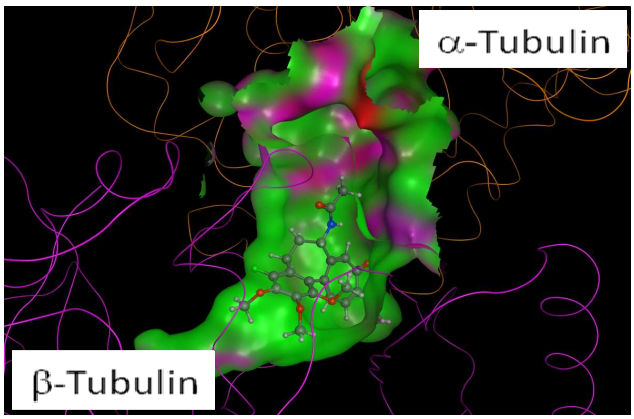
Docking study.

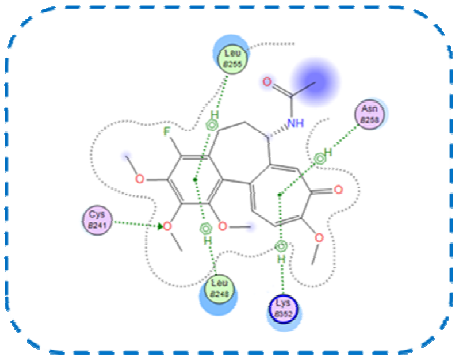
An X-ray crystal structure of a colchicine derivative (DAMA colchicine) with tubulin protein was available from the PDB using accession code 1SA0. We performed docking study with Glide based on this PDB data (Figure S1 (a) - (d)).

Figure S1. Docking study of colchicine (**1**), 4-fluorocolchicine (**3**) and 4-hydroxycolchicine (**8**) with tubulin protein.

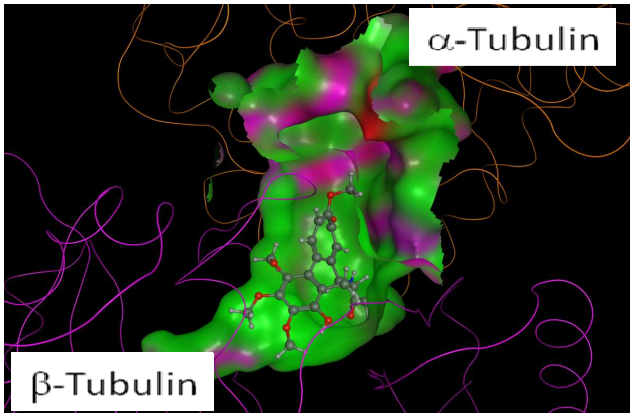


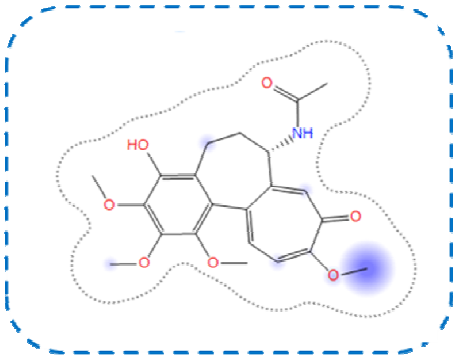
Colchicine (**1**) (Score: -8.23 kcal/ mol)





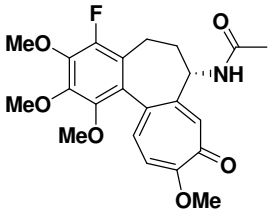
4-Fluorocolchicine(**3**) (Score: -8.38 kcal/ mol)





4-Hydroxycolchicine (**8**) (Score: -6.59 kcal/ mol)

^{1}H and ^{13}C-NMR spectra for **3**



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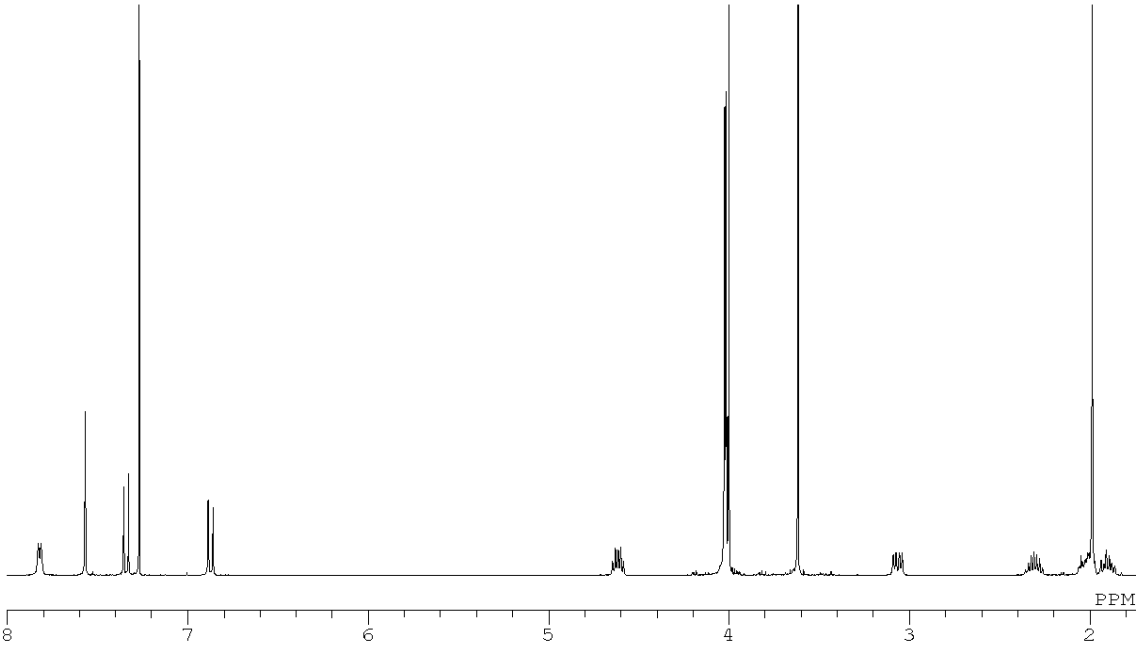
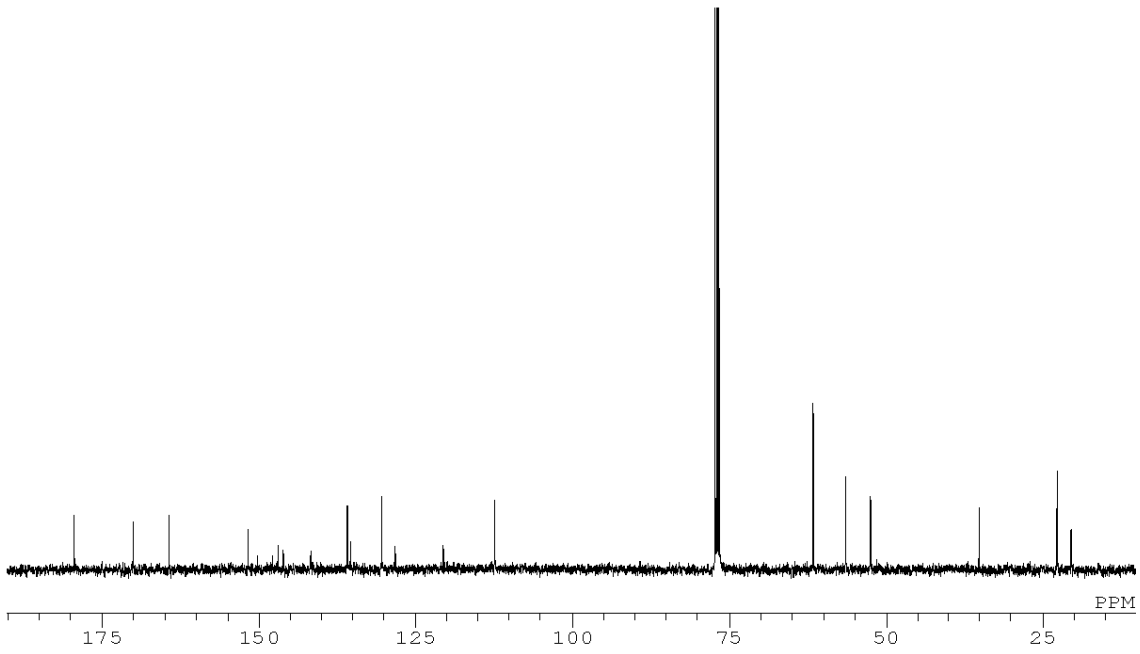
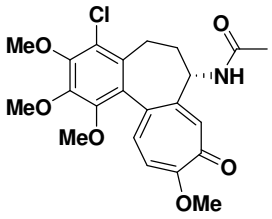




^{1}H and ^{13}C-NMR spectra for **4**



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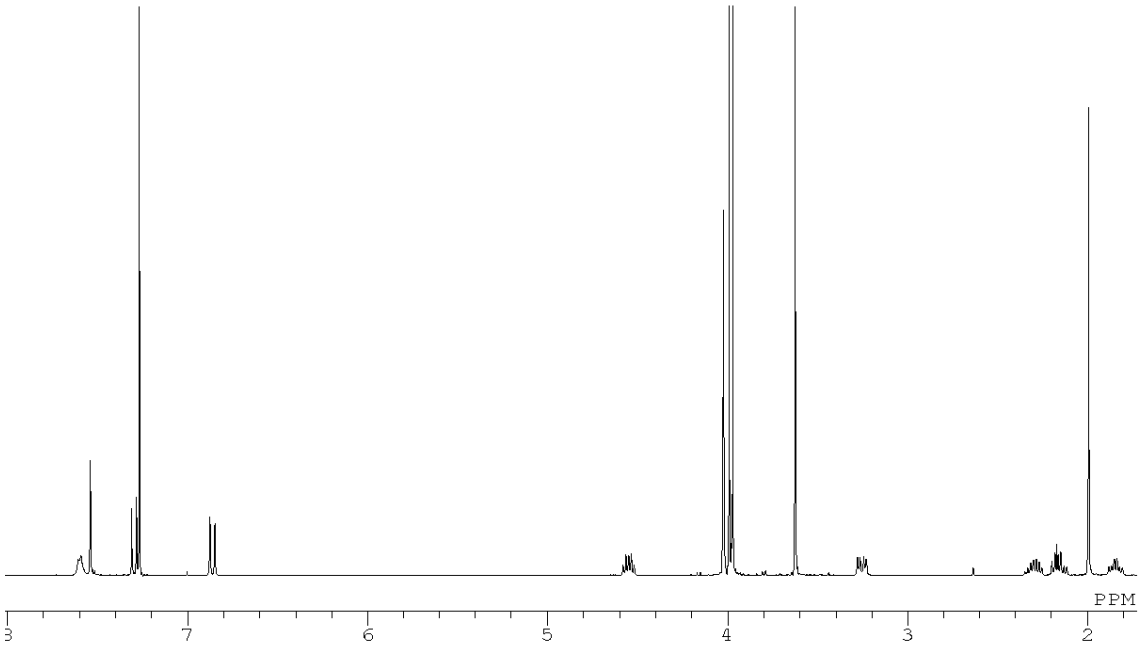
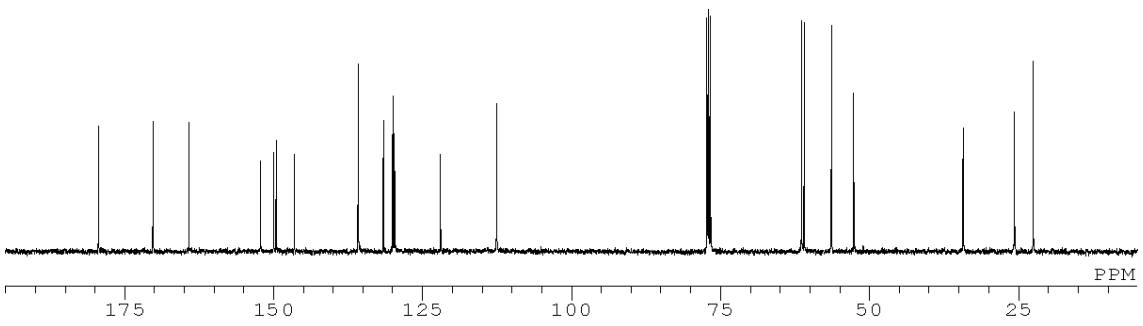
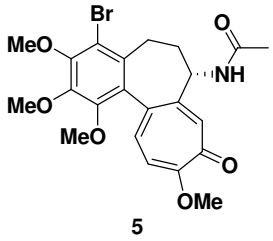




^{1}H and ^{13}C-NMR spectra for **5**



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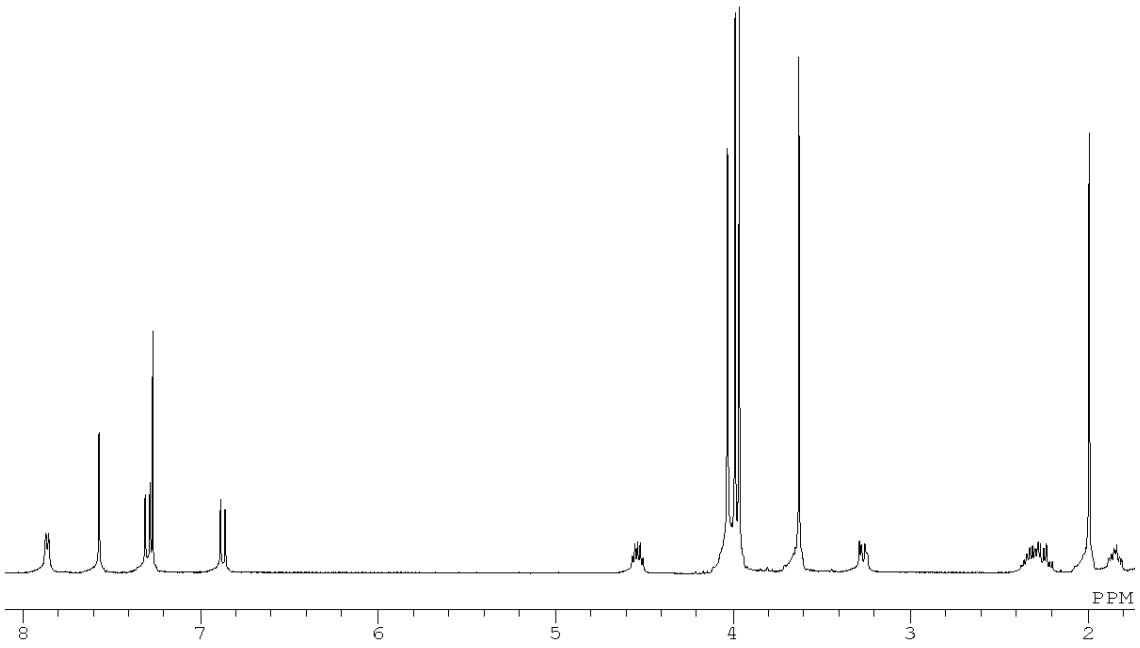
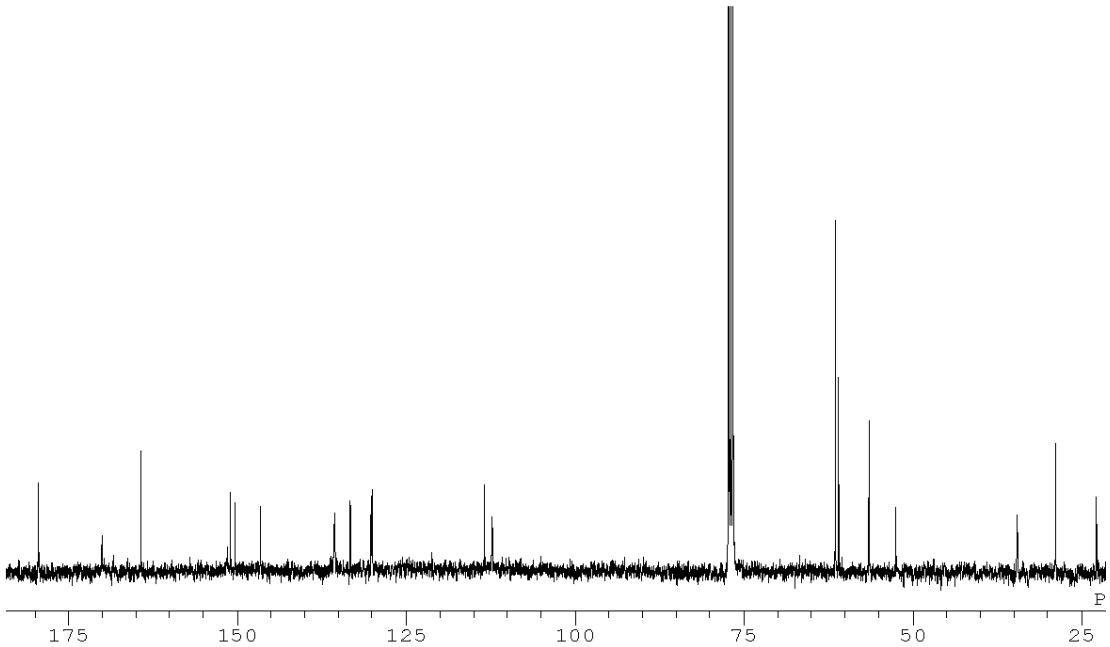
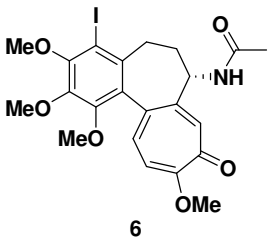
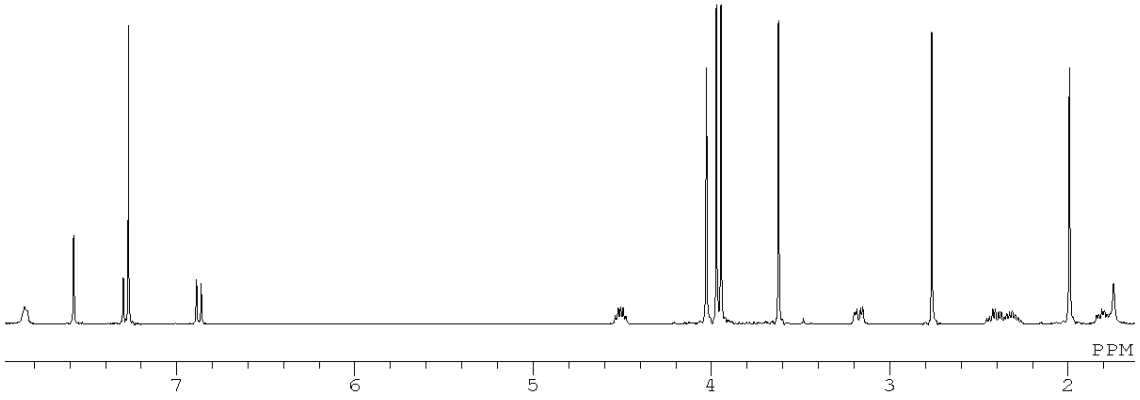


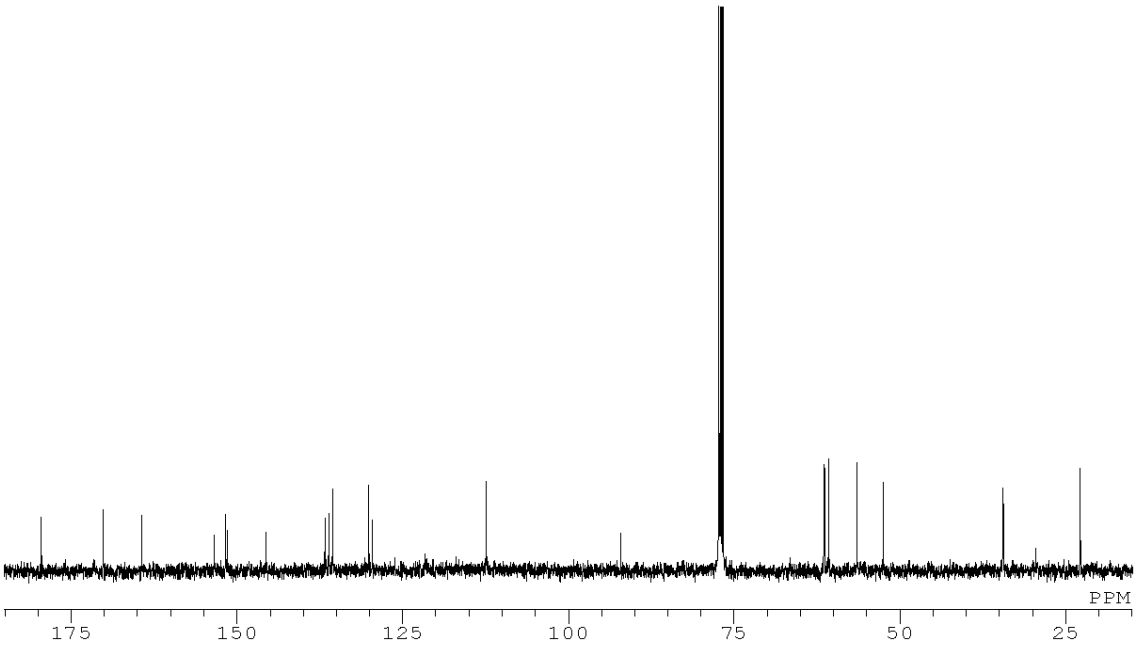


^{1}H and ^{13}C-NMR spectra for **6**

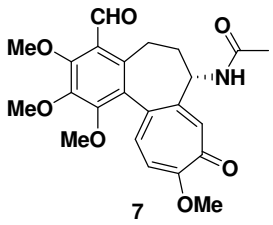


6





^{1}H and ^{13}C-NMR spectra for **7**



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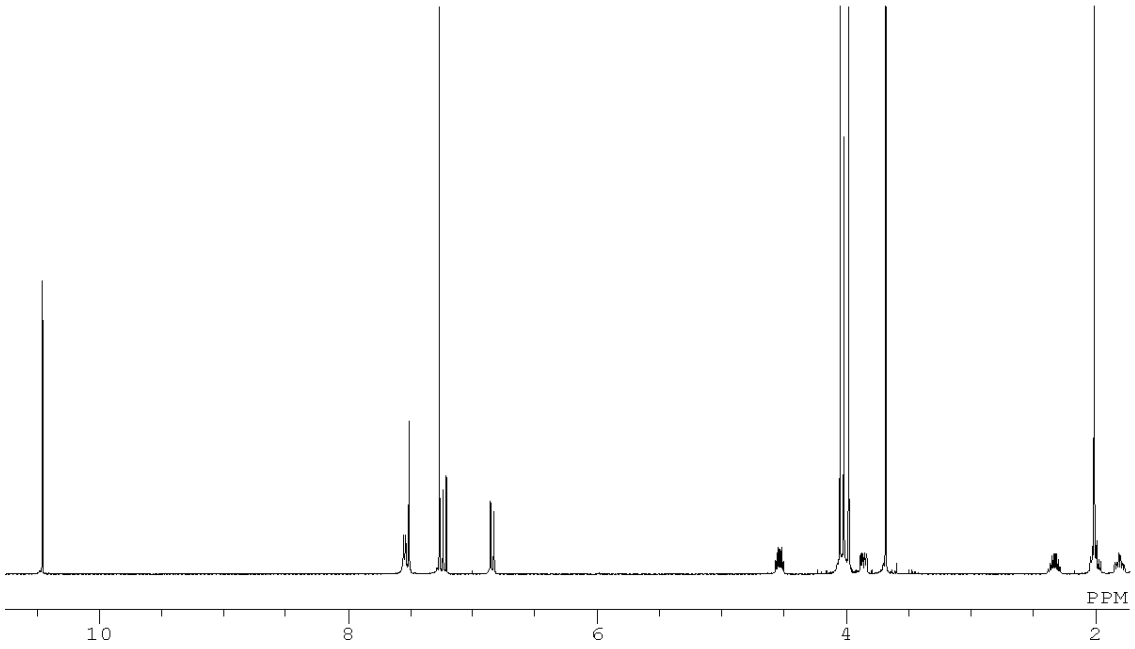
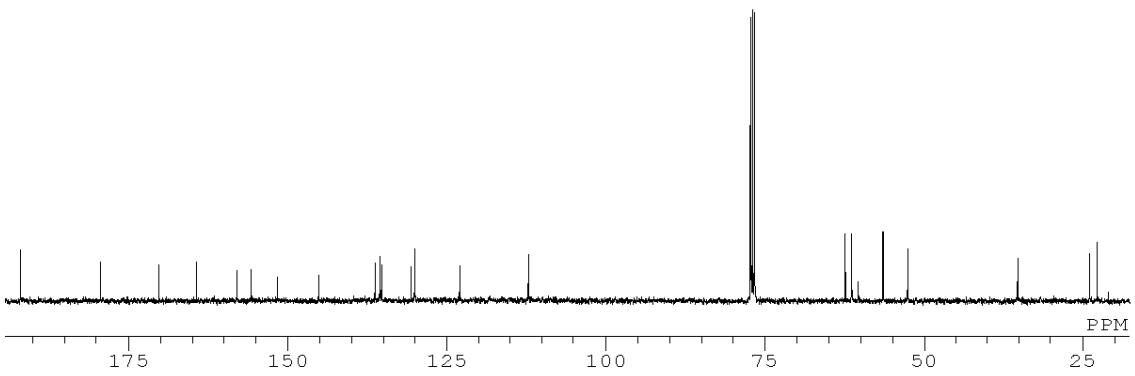
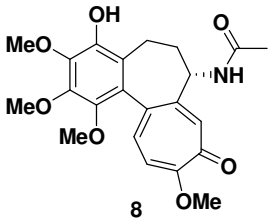




^{1}H and ^{13}C-NMR spectra for **8**



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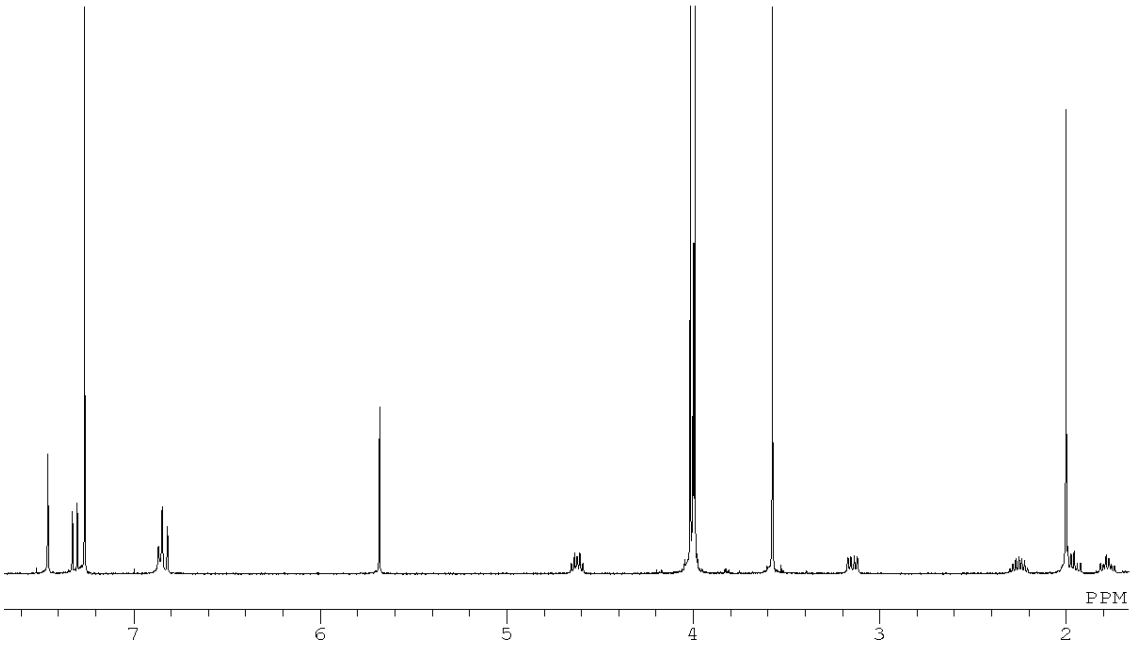
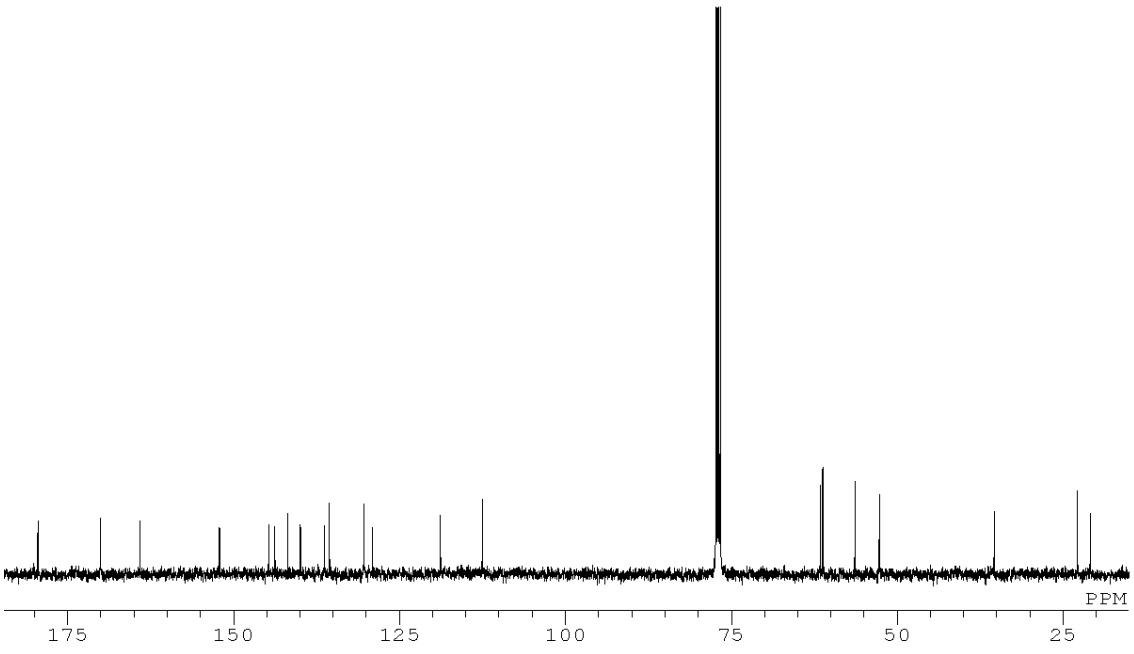
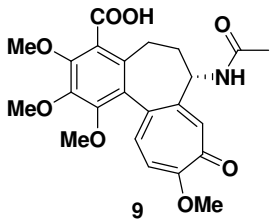




^{1}H and ^{13}C-NMR spectra for **9**



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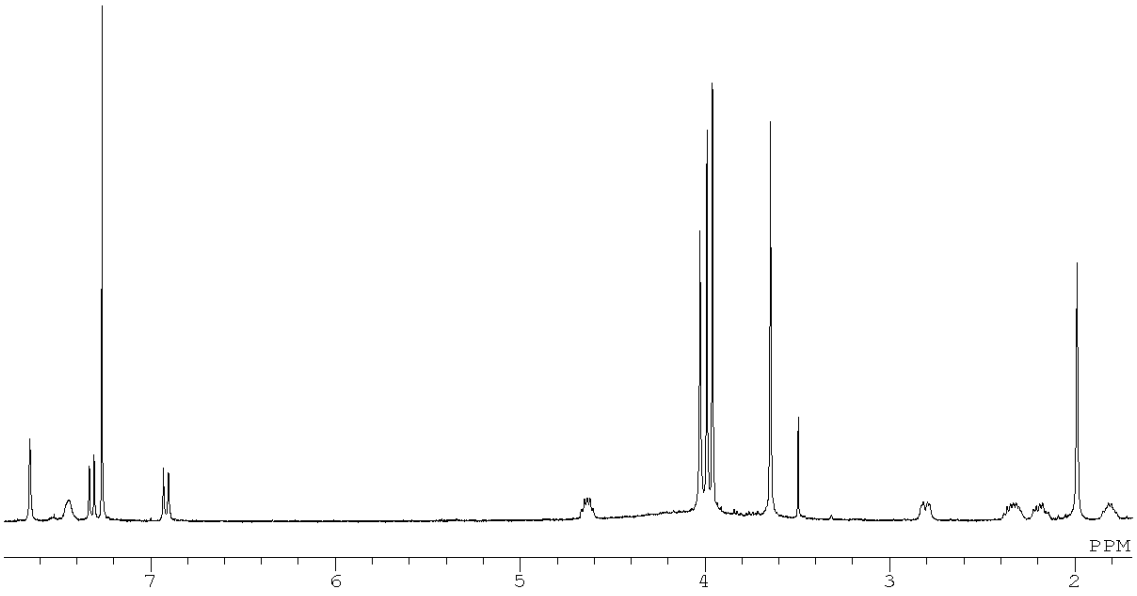
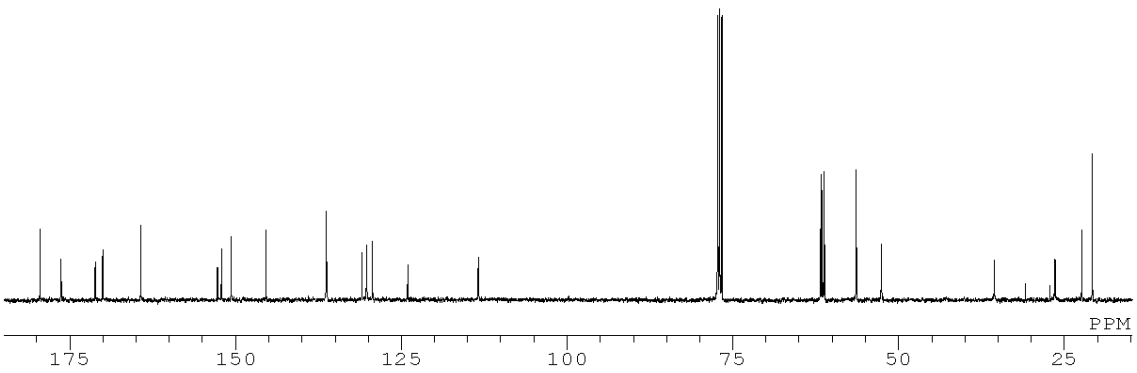
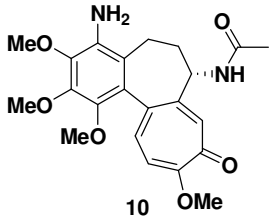




^{1}H and ^{13}C-NMR spectra for **10**



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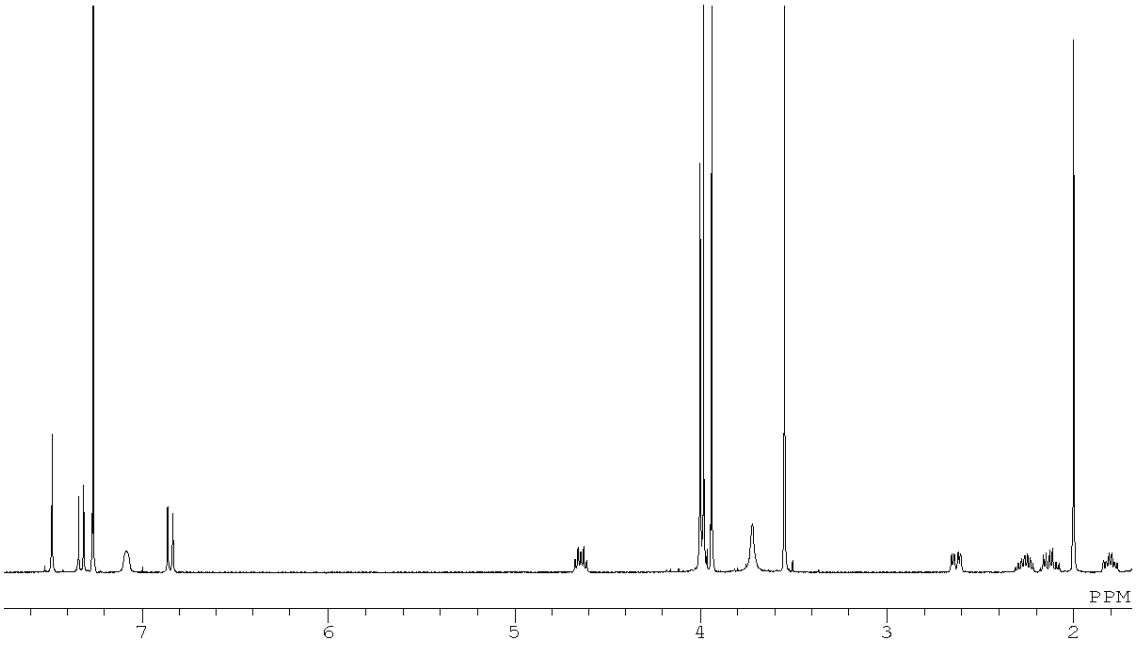
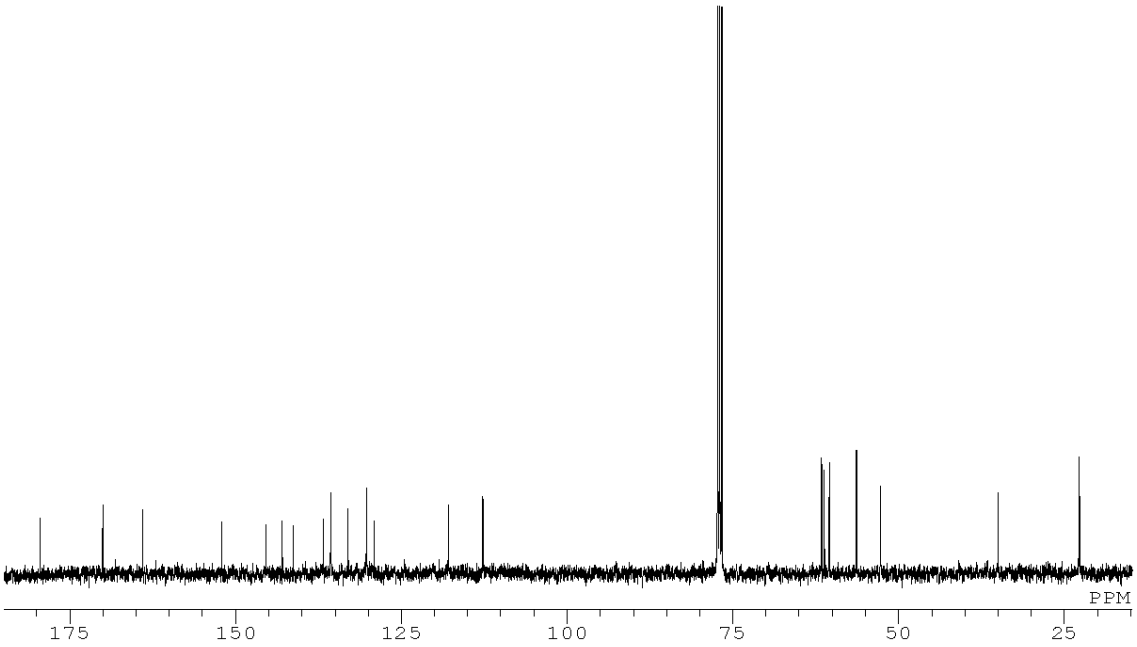
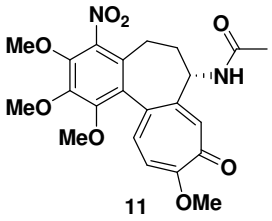




^{1}H and ^{13}C-NMR spectra for **11**



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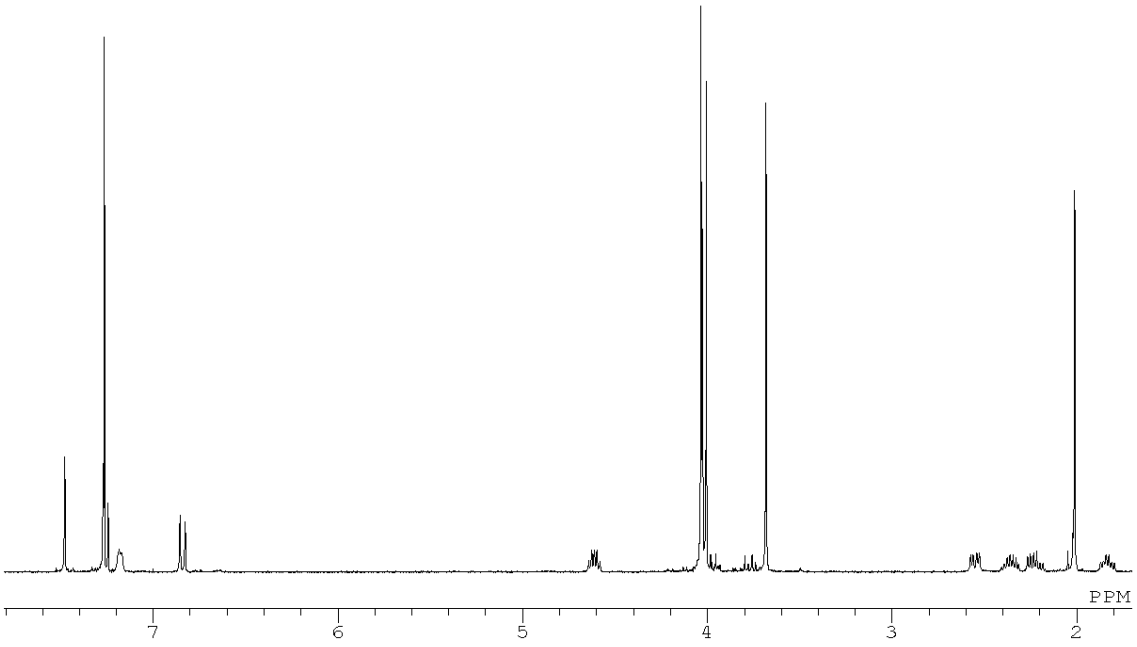
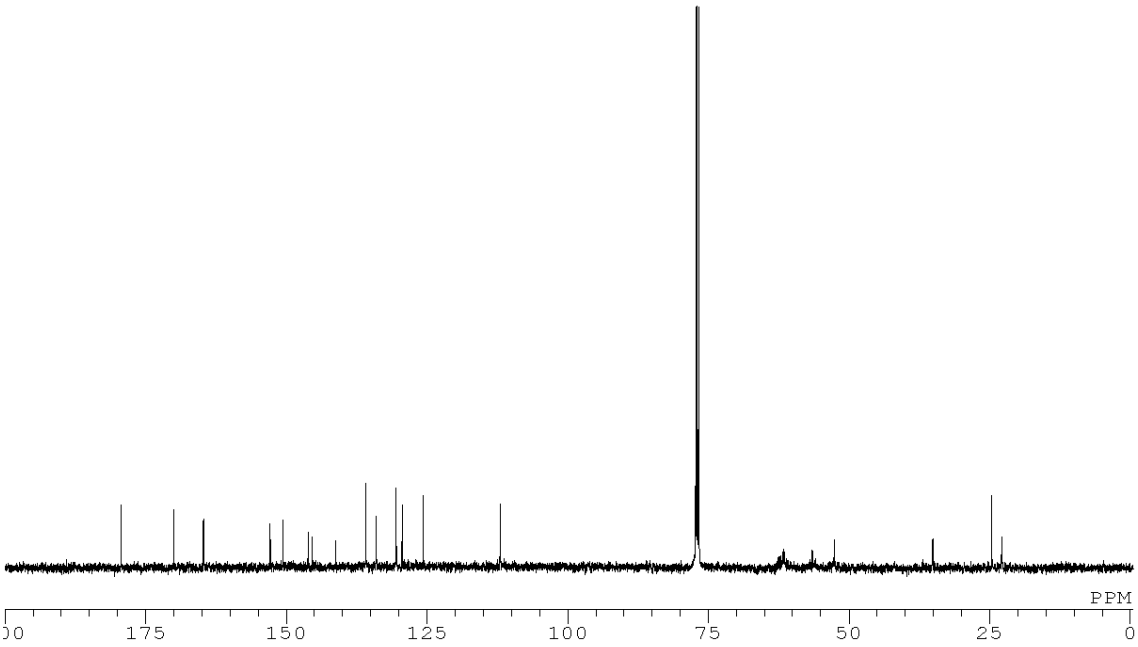
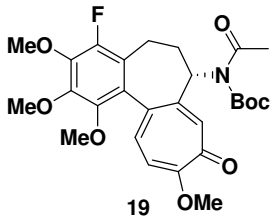




^{1}H and ^{13}C-NMR spectra for **19**



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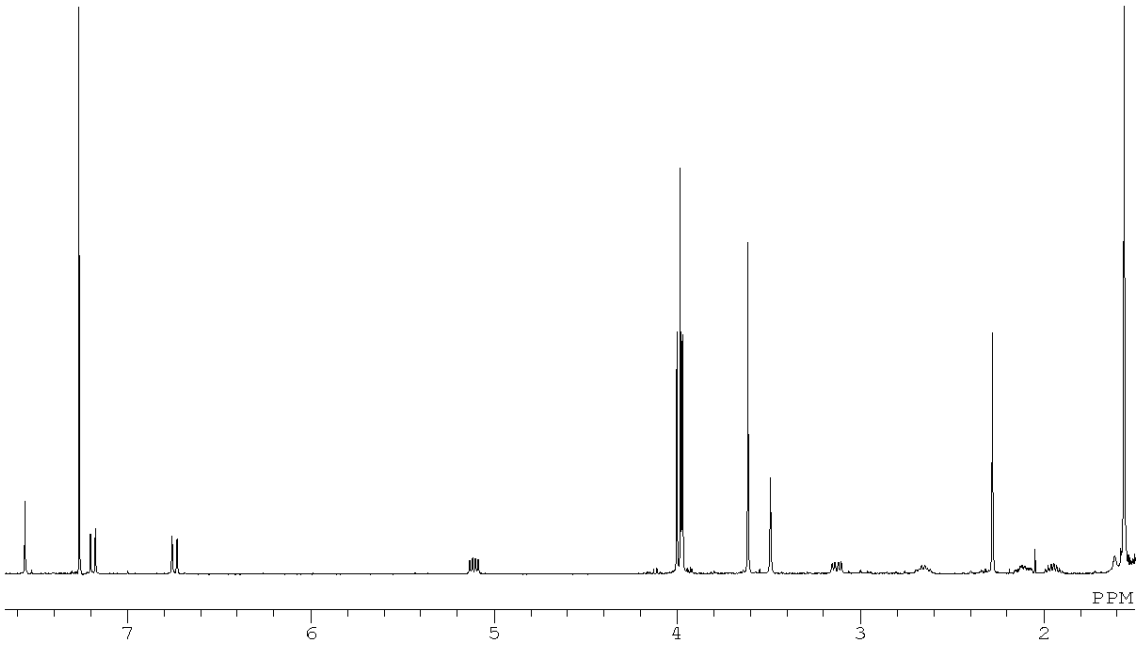
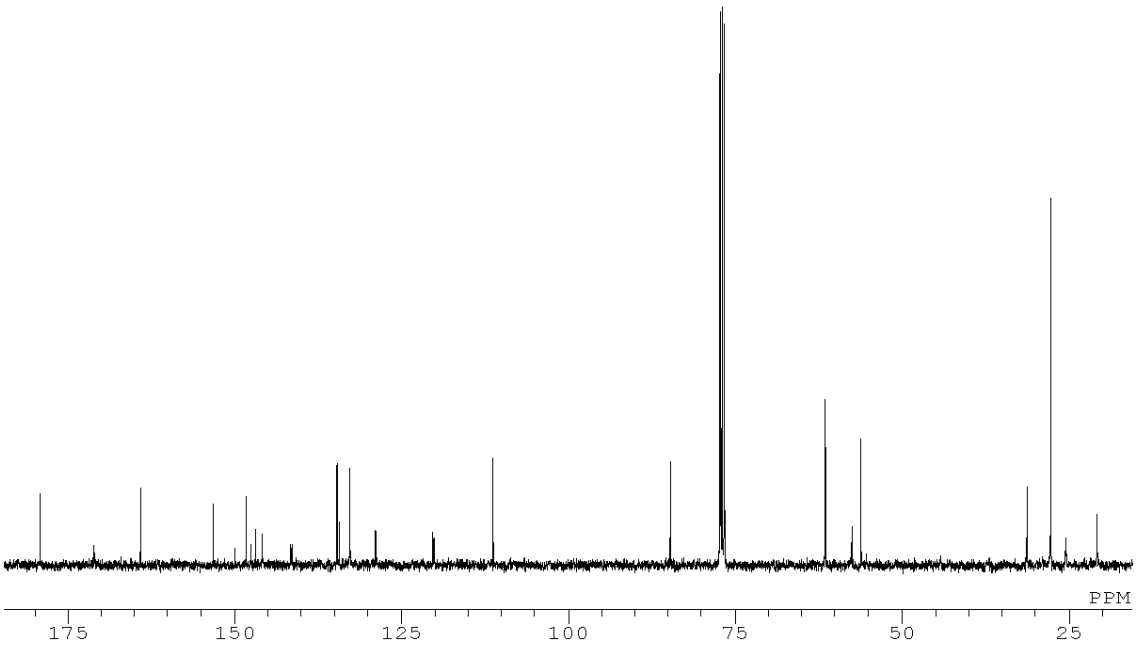
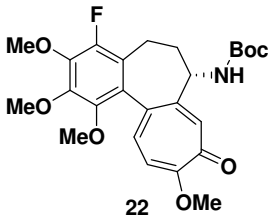




^{1}H and ^{13}C-NMR spectra for **22**



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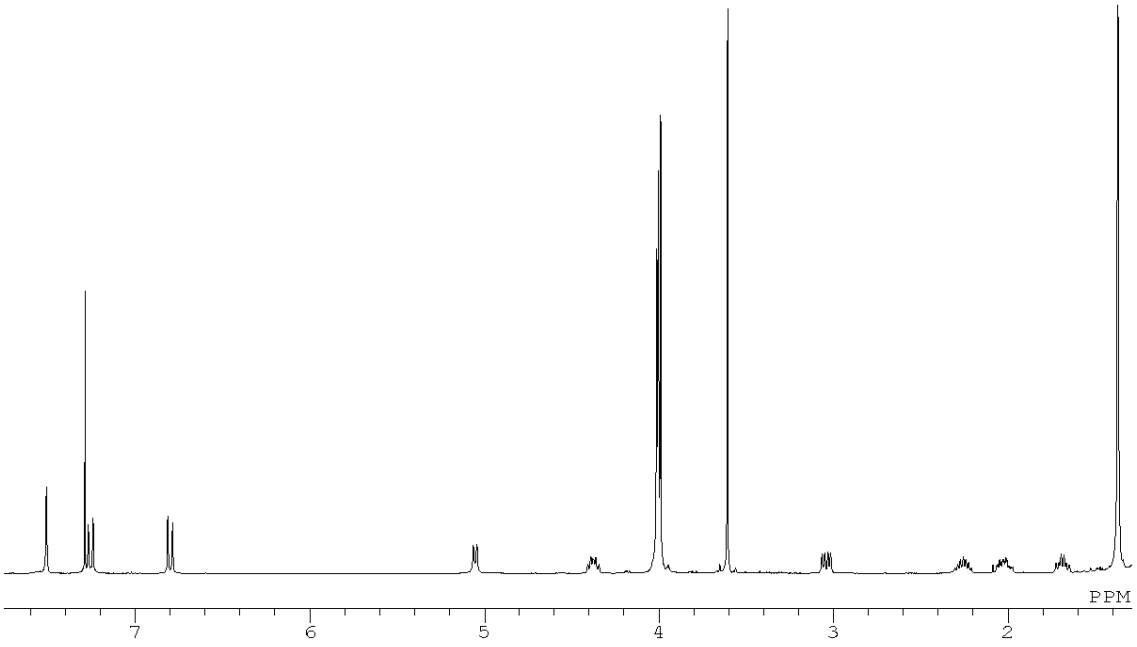
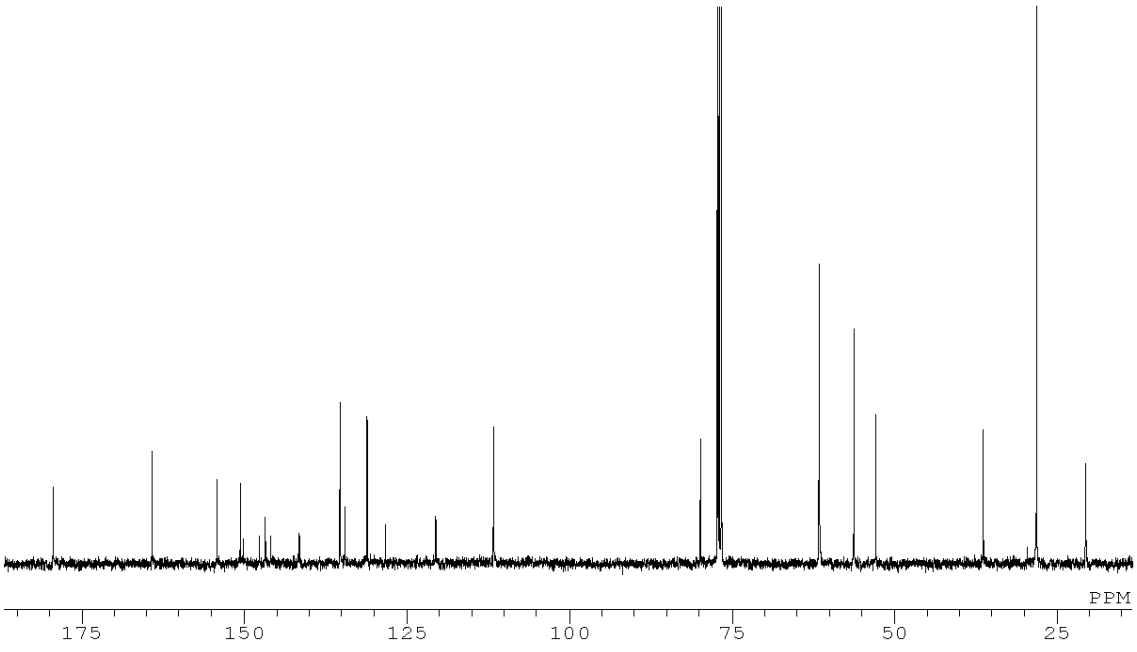
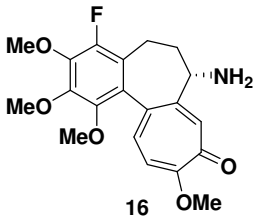




^{1}H and ^{13}C-NMR spectra for **16**



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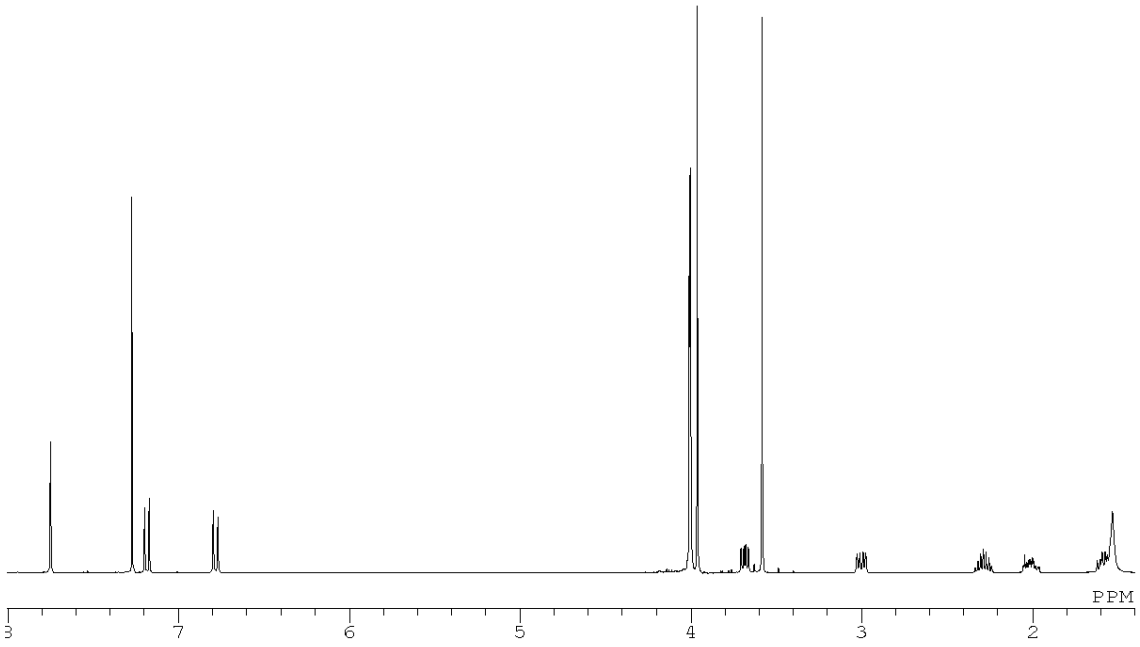
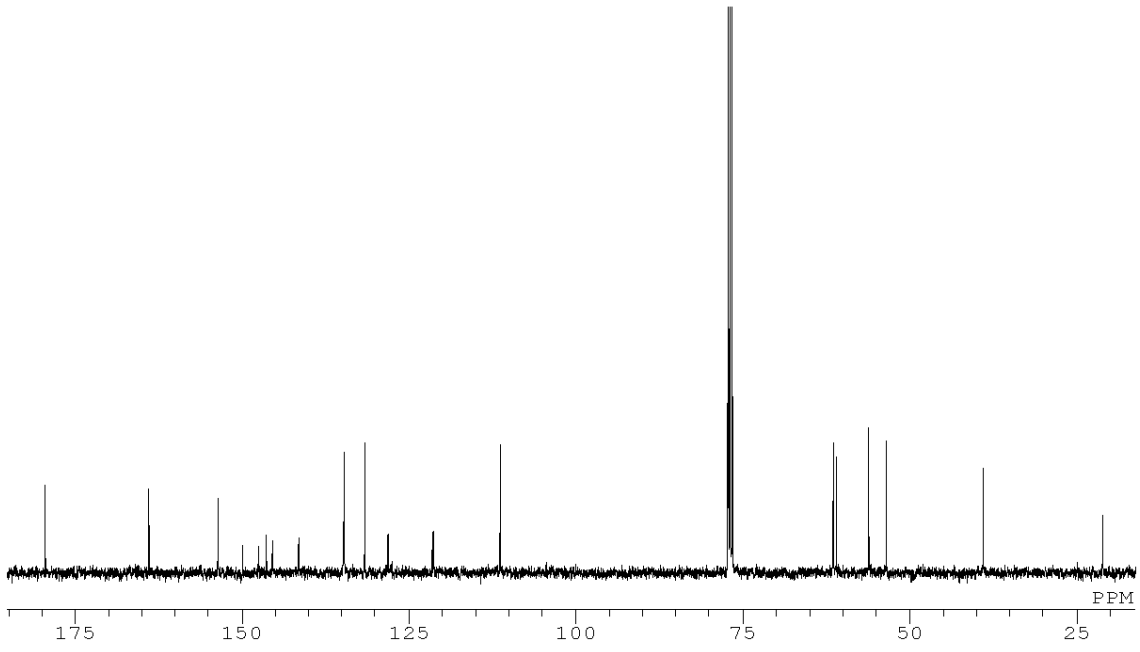
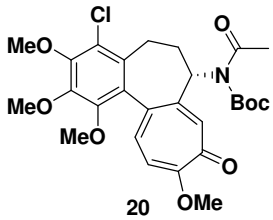




^{1}H and ^{13}C-NMR spectra for **20**



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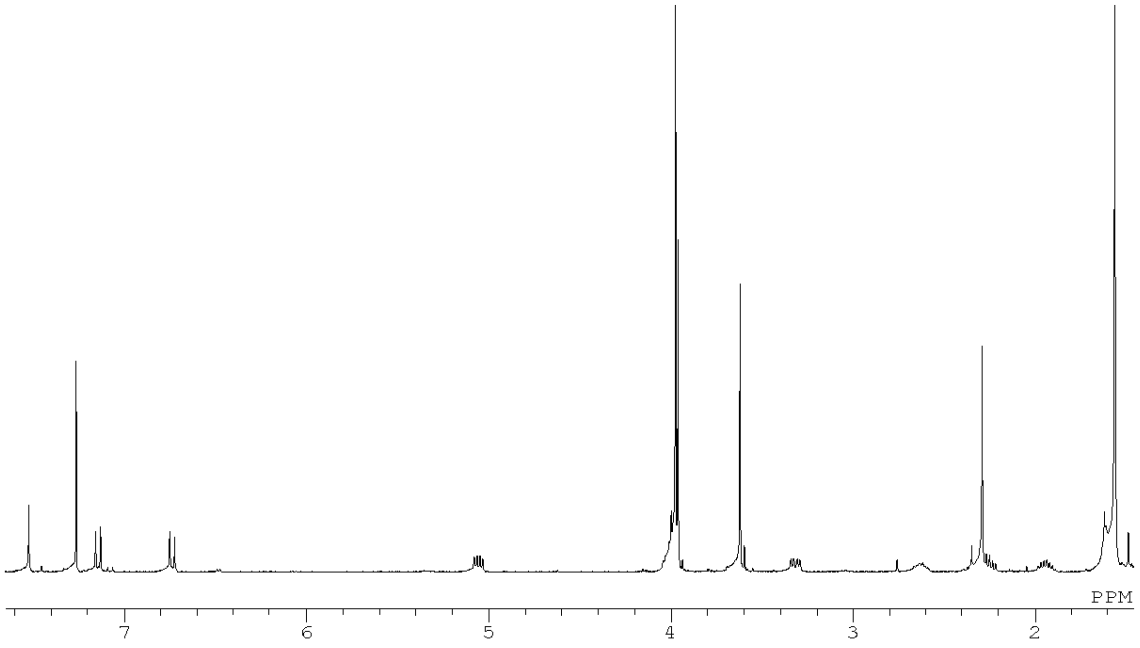
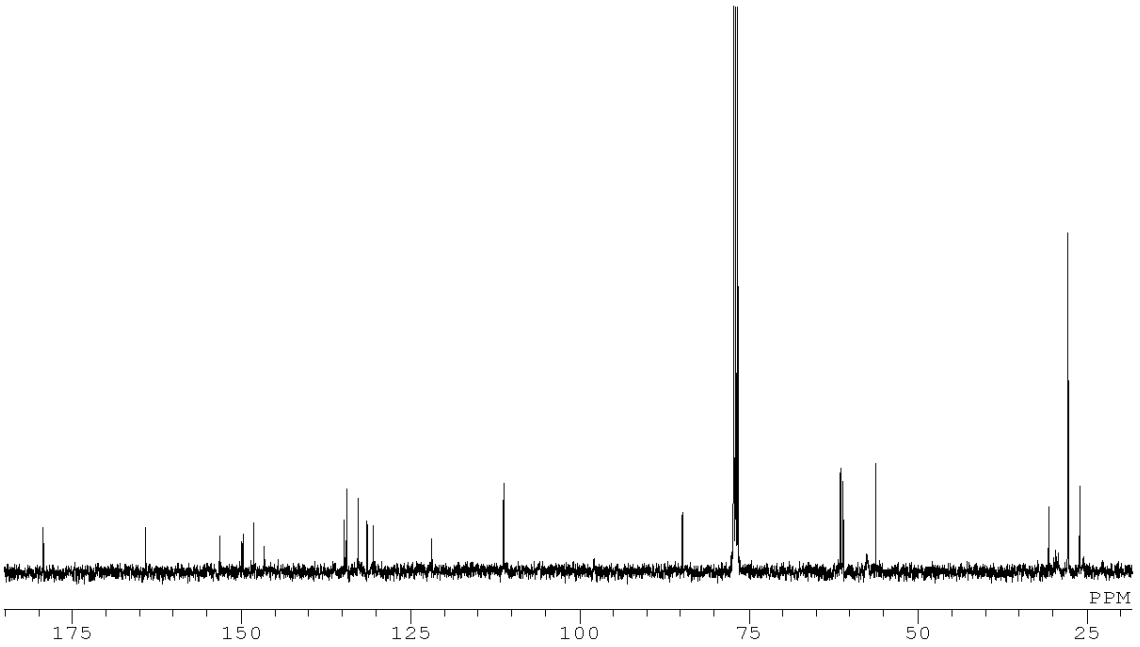
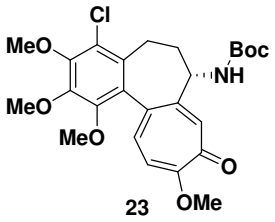




^{1}H and ^{13}C-NMR spectra for **23**



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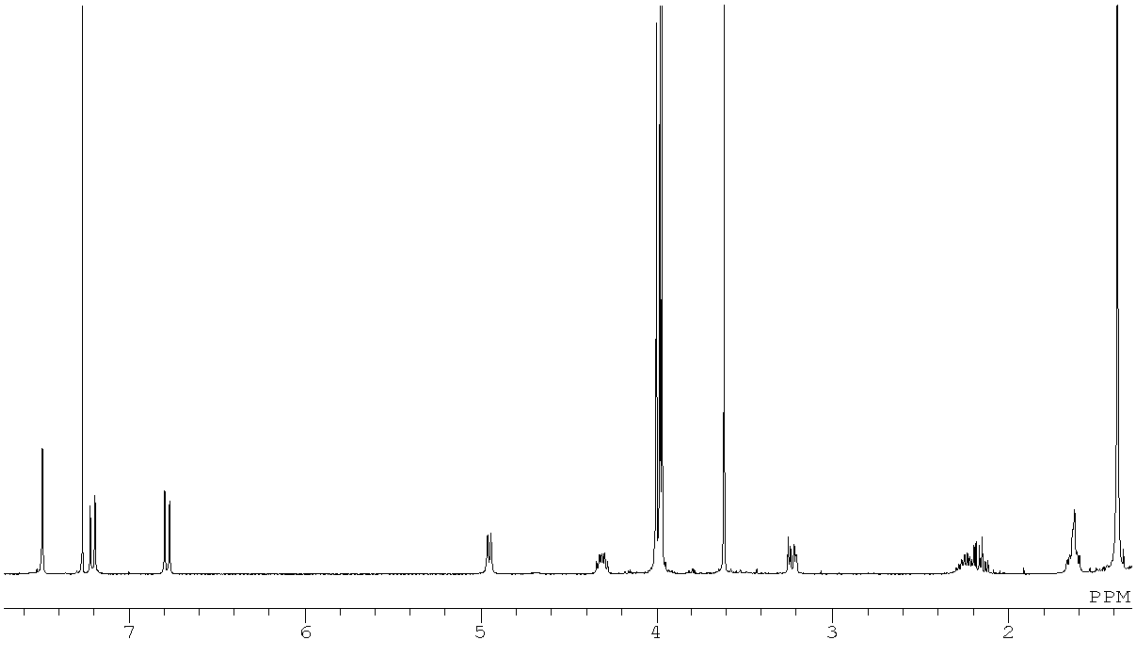
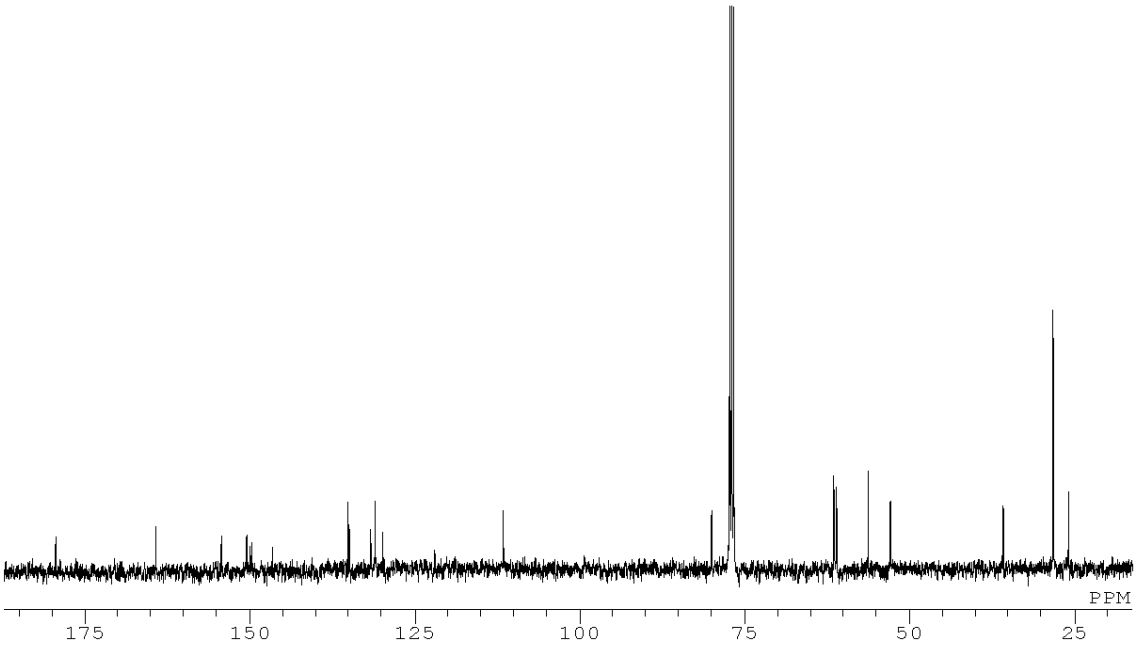
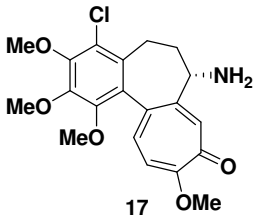




^{1}H and ^{13}C-NMR spectra for **17**



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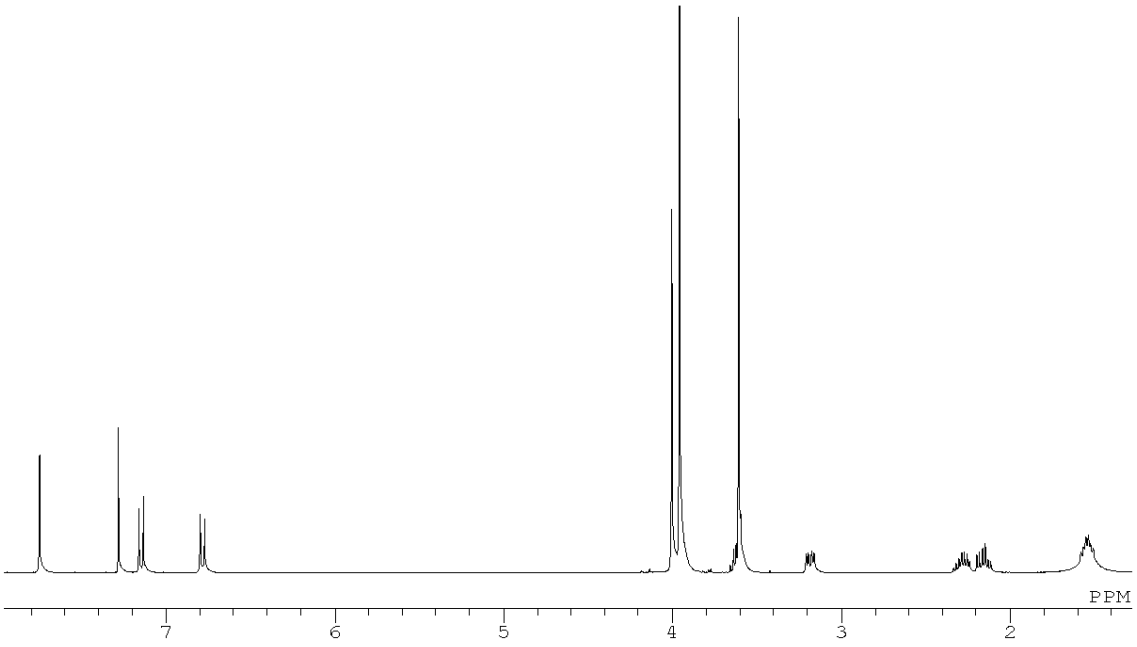
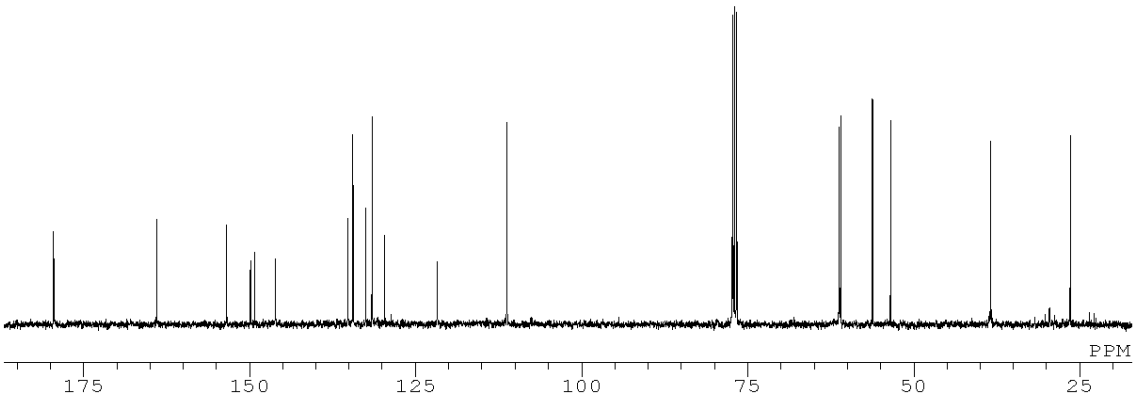
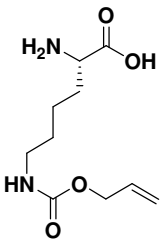




^{1}H and ^{13}C-NMR spectra for **25**



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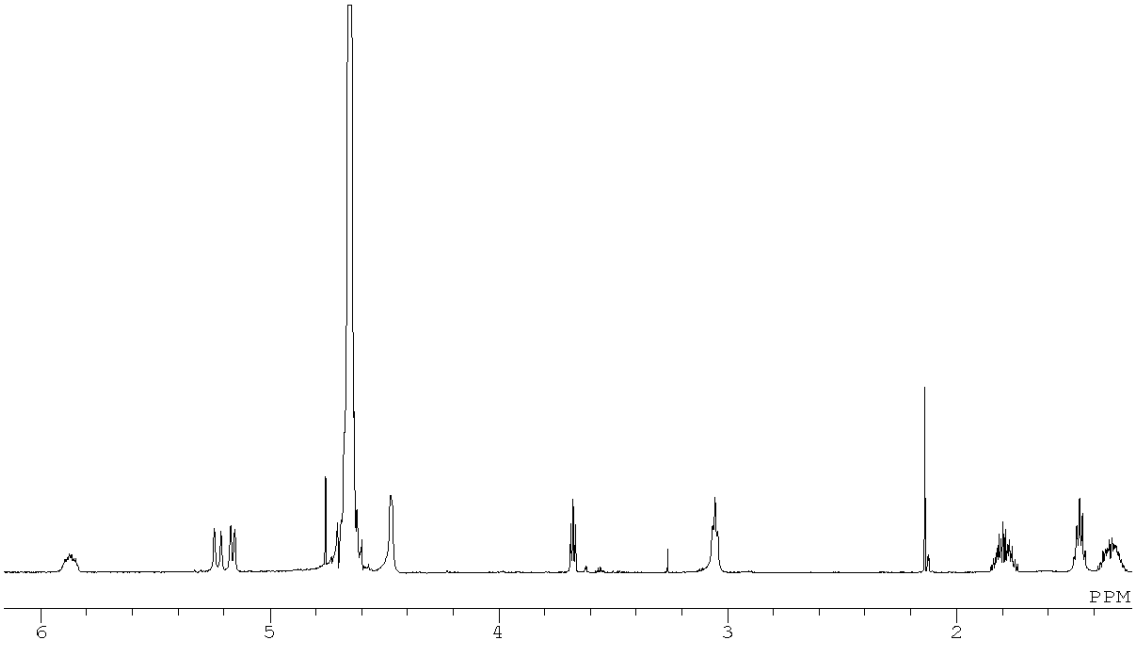
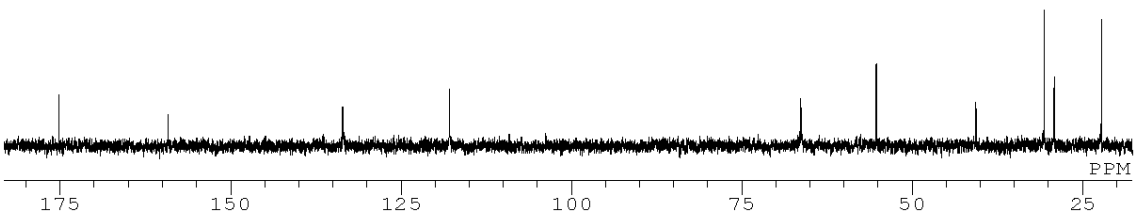
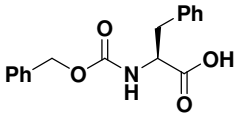
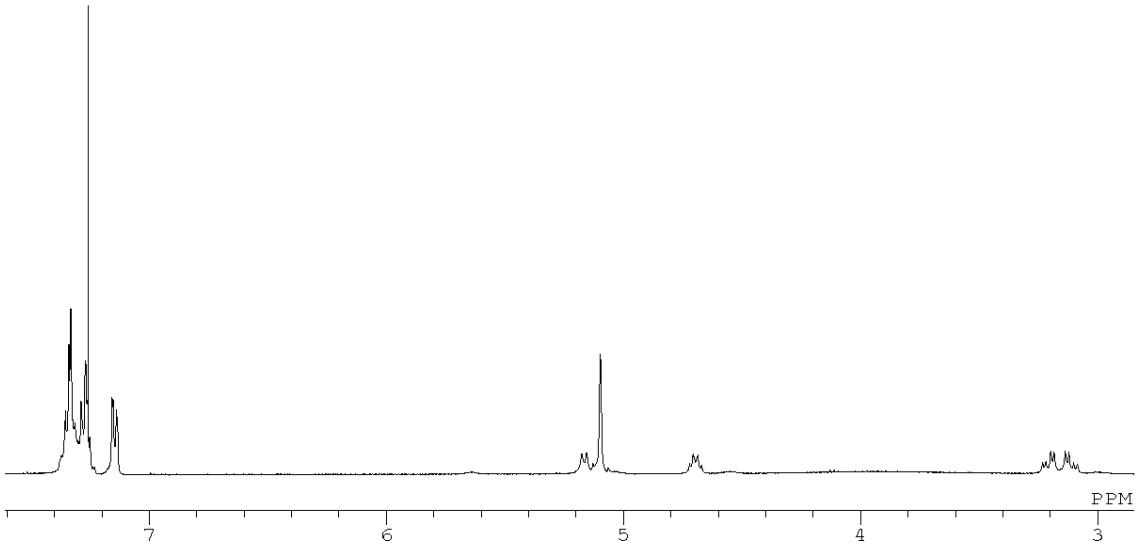


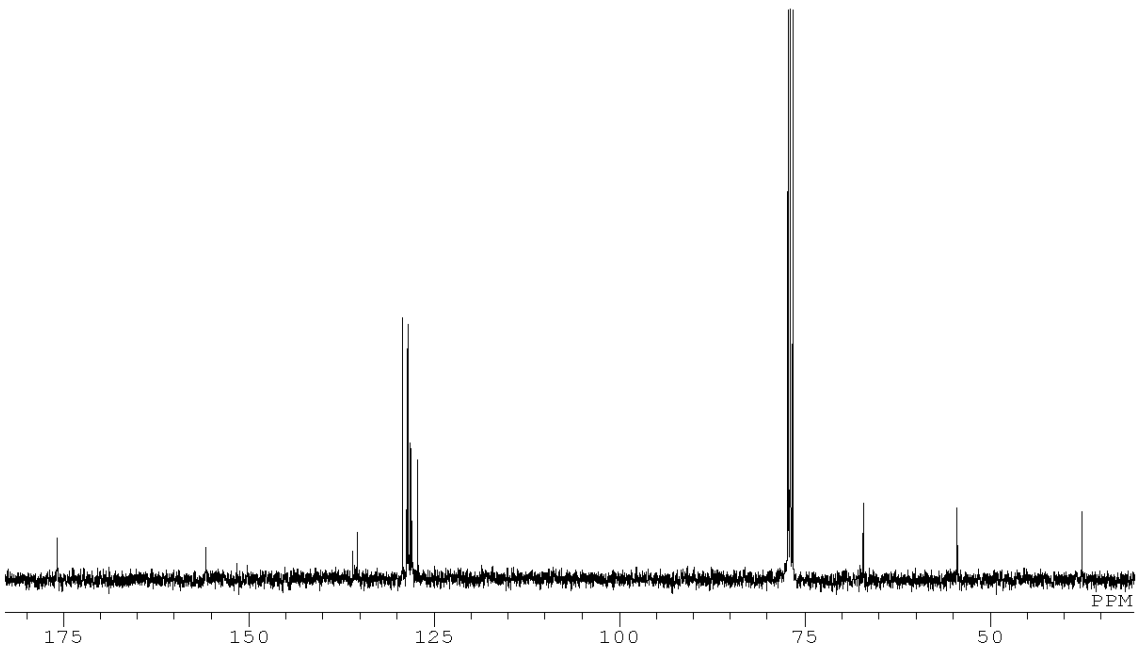


^{1}H and ^{13}C-NMR spectra for **27**

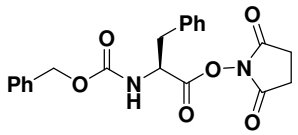


27

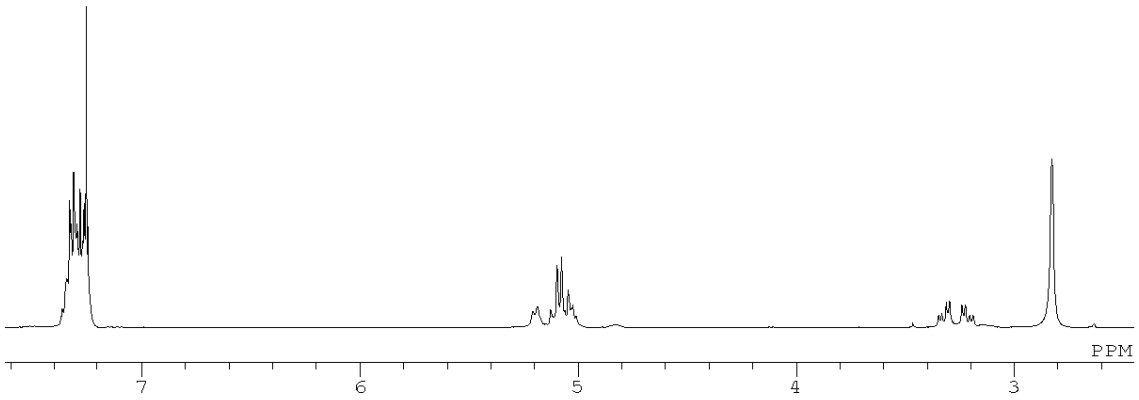


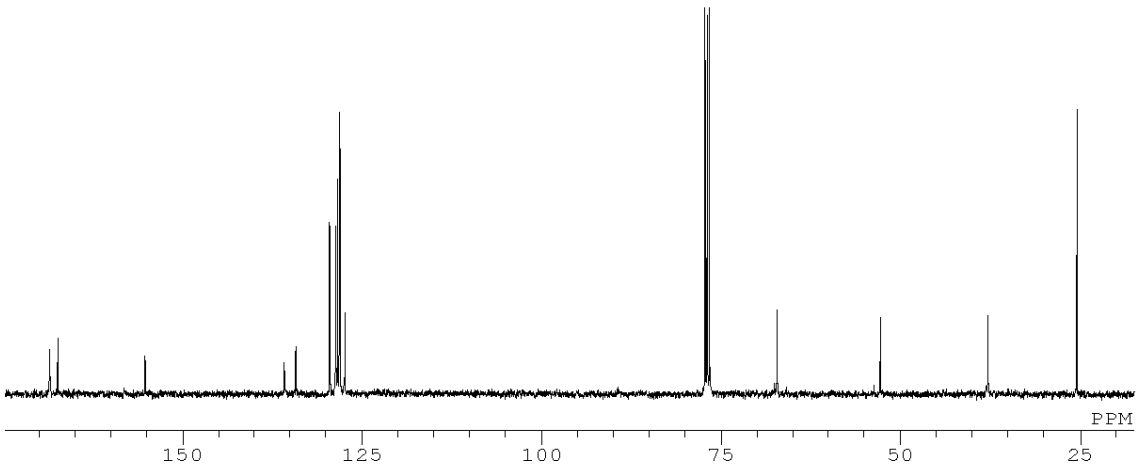


^{1}H and ^{13}C-NMR spectra for **28**

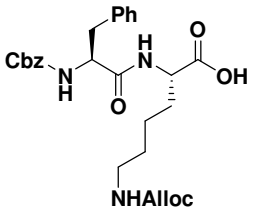


28

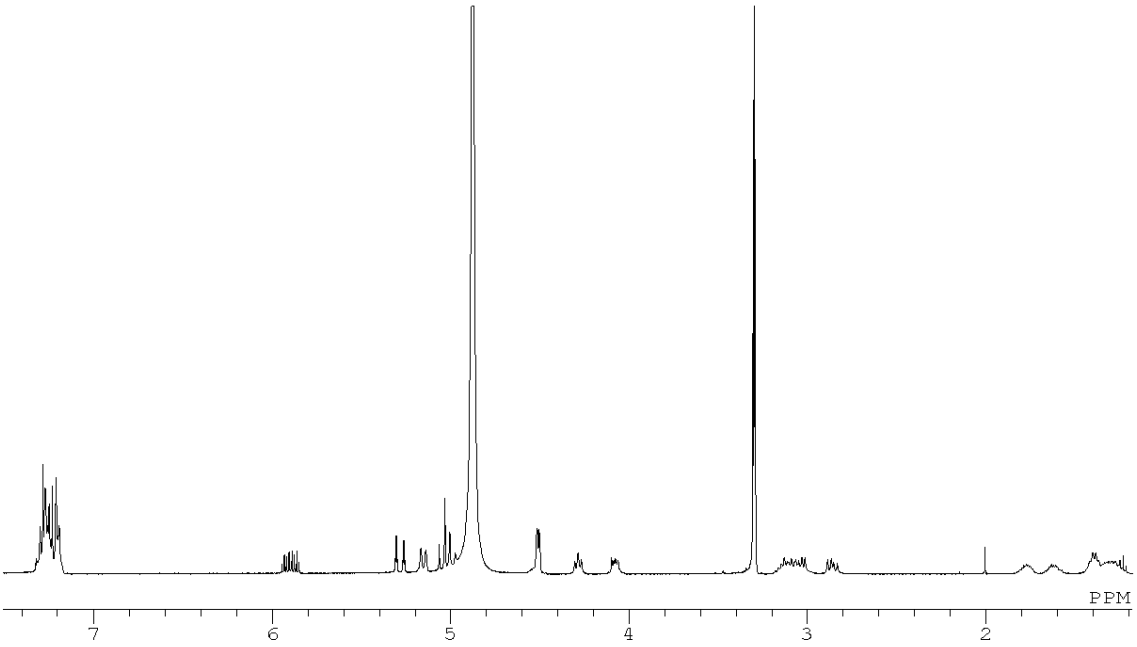


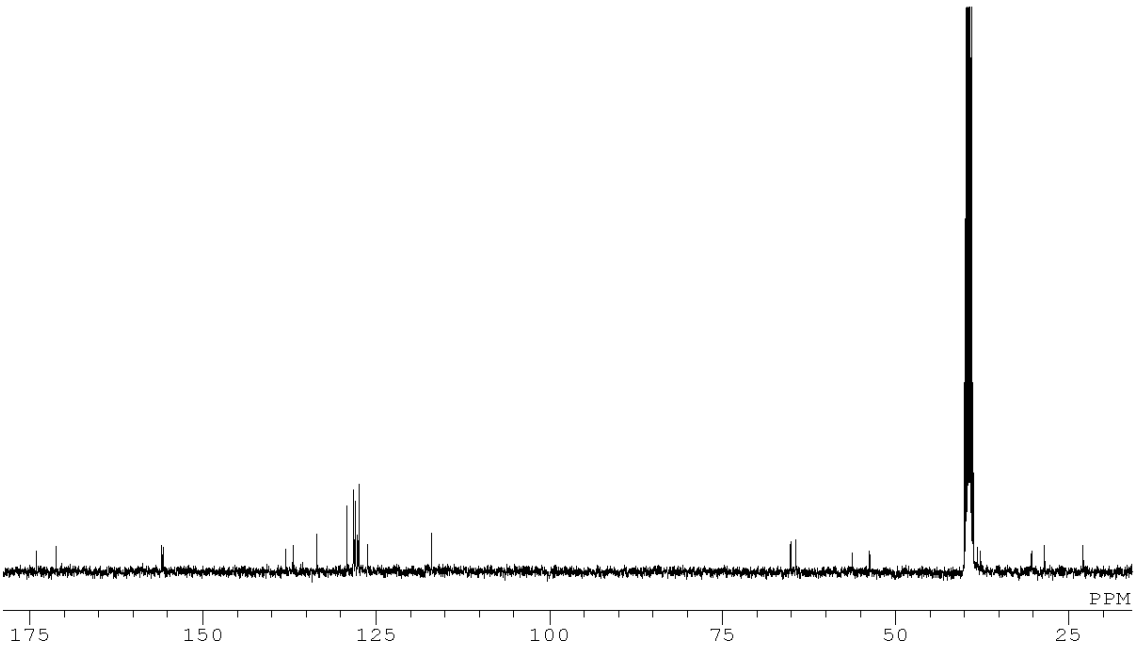


^{1}H and ^{13}C-NMR spectra for **29**

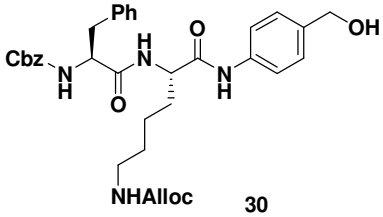


29

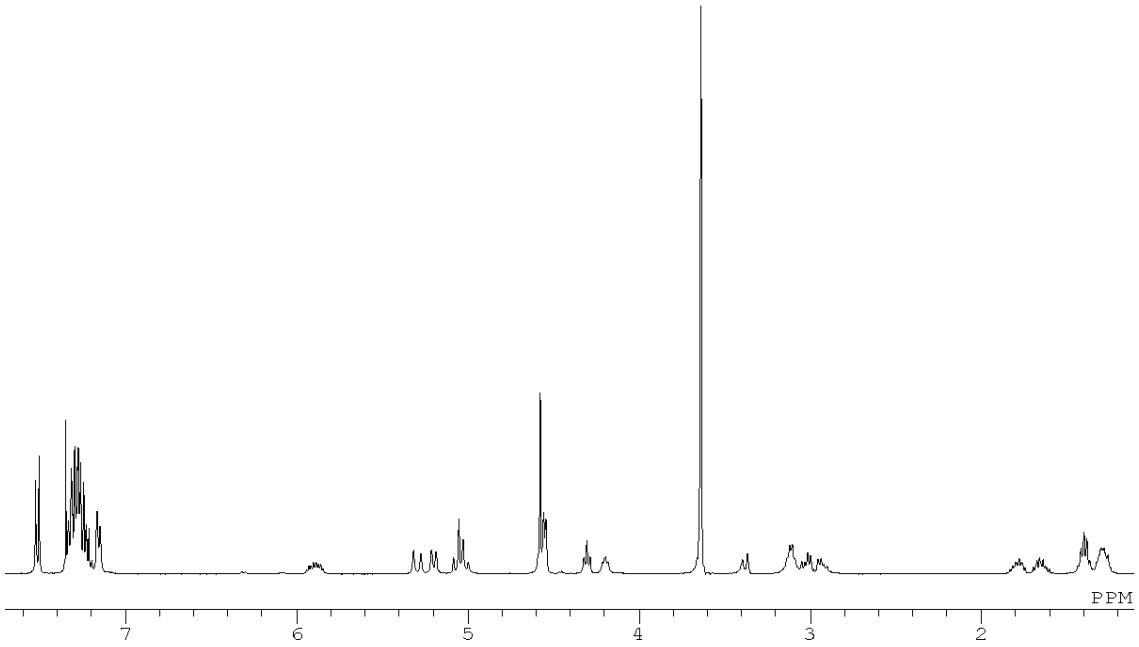


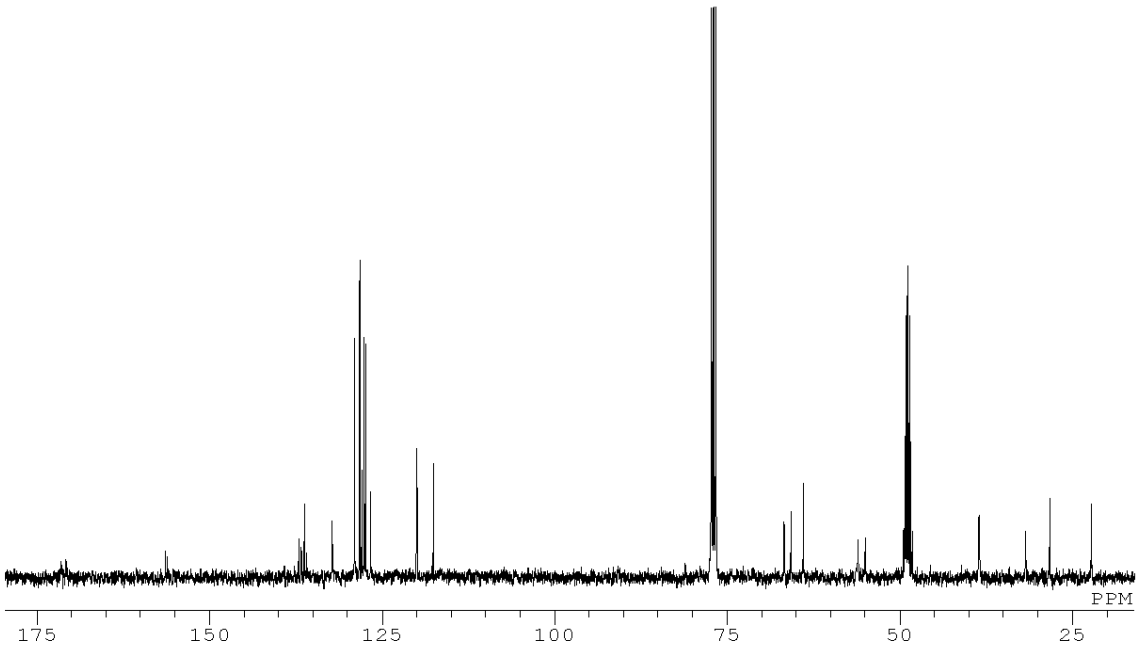


^{1}H and ^{13}C-NMR spectra for **30**

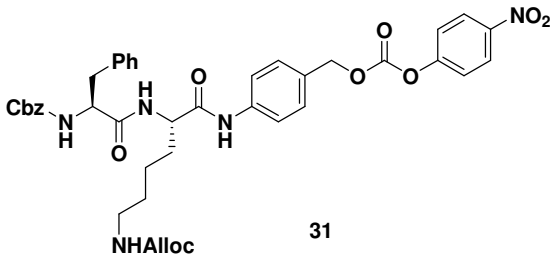


30





^{1}H and ^{13}C-NMR spectra for **31**



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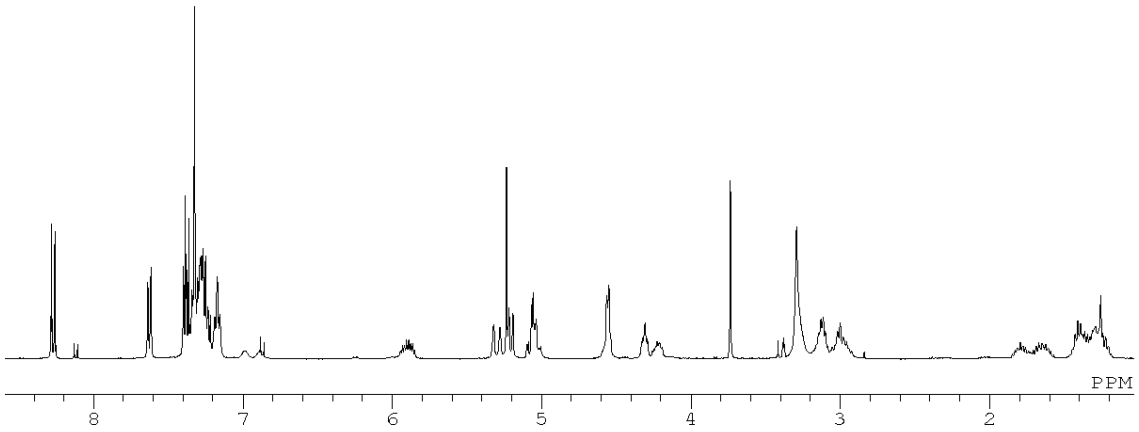
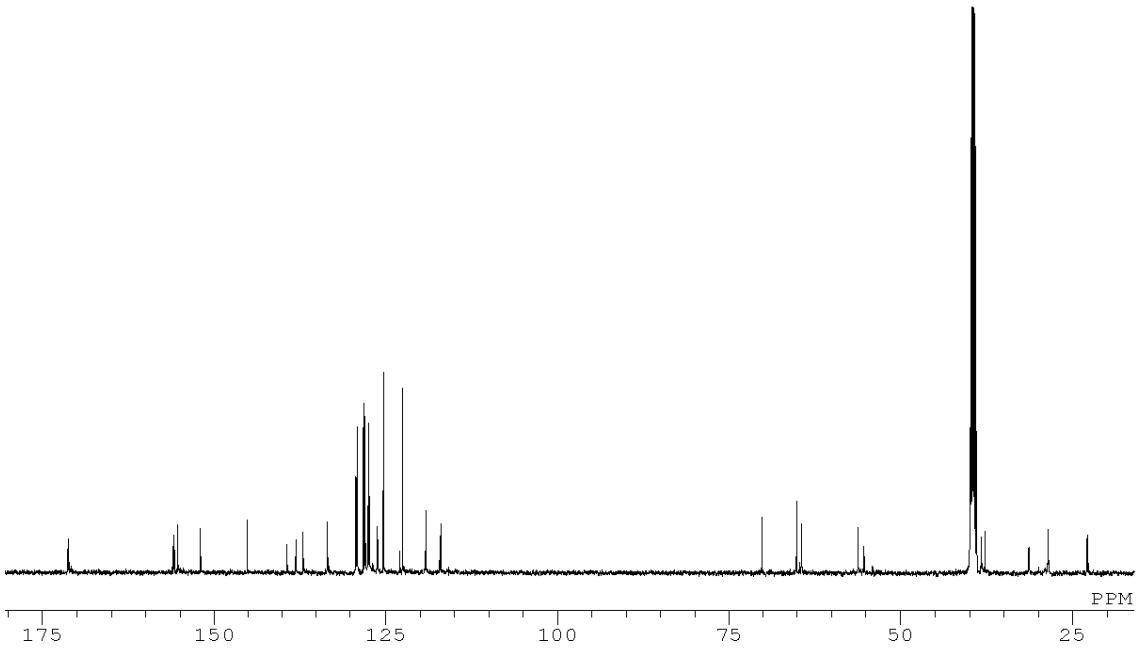
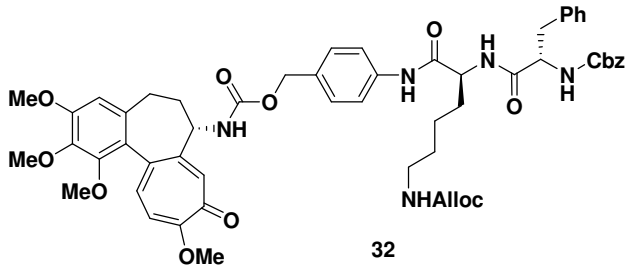
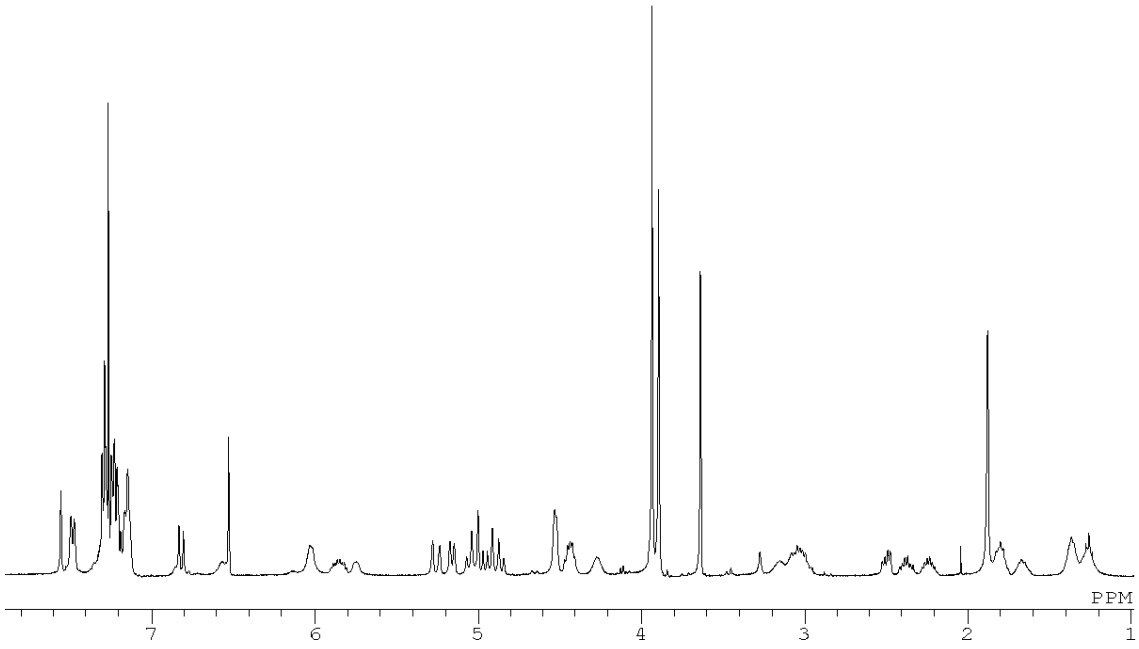


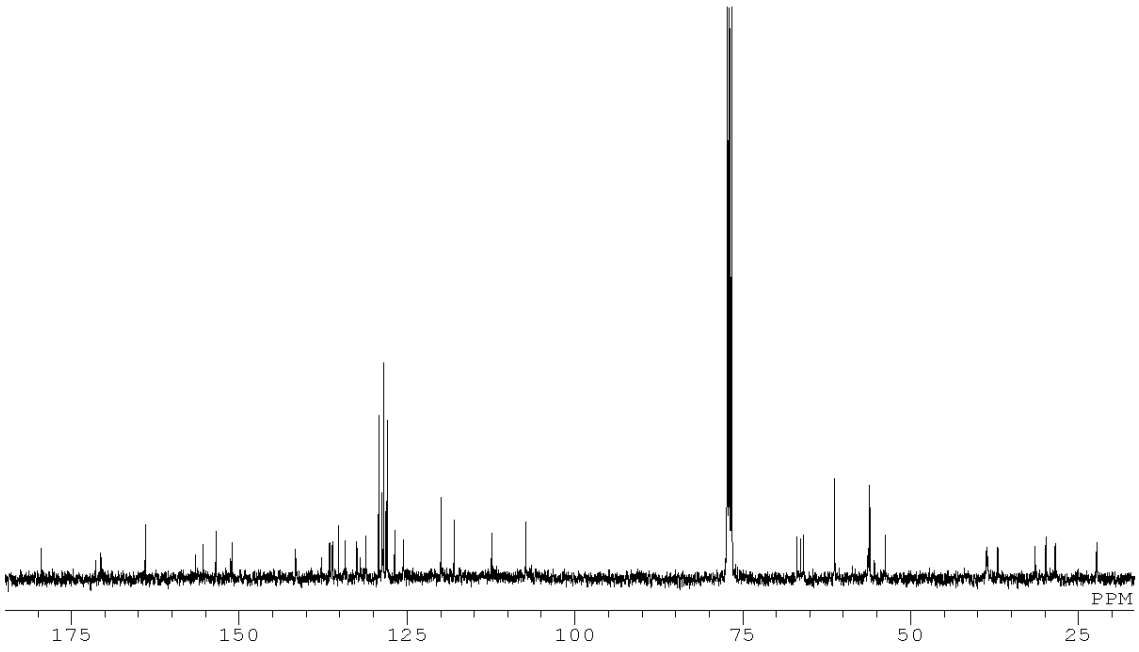


^{1}H and ^{13}C-NMR spectra for **32**

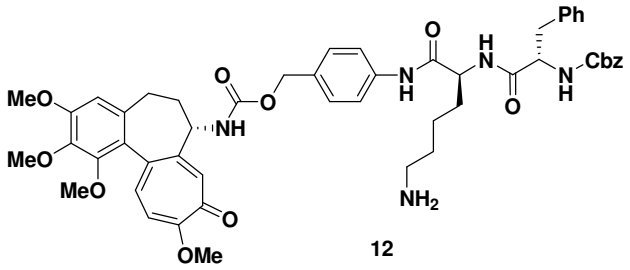


32

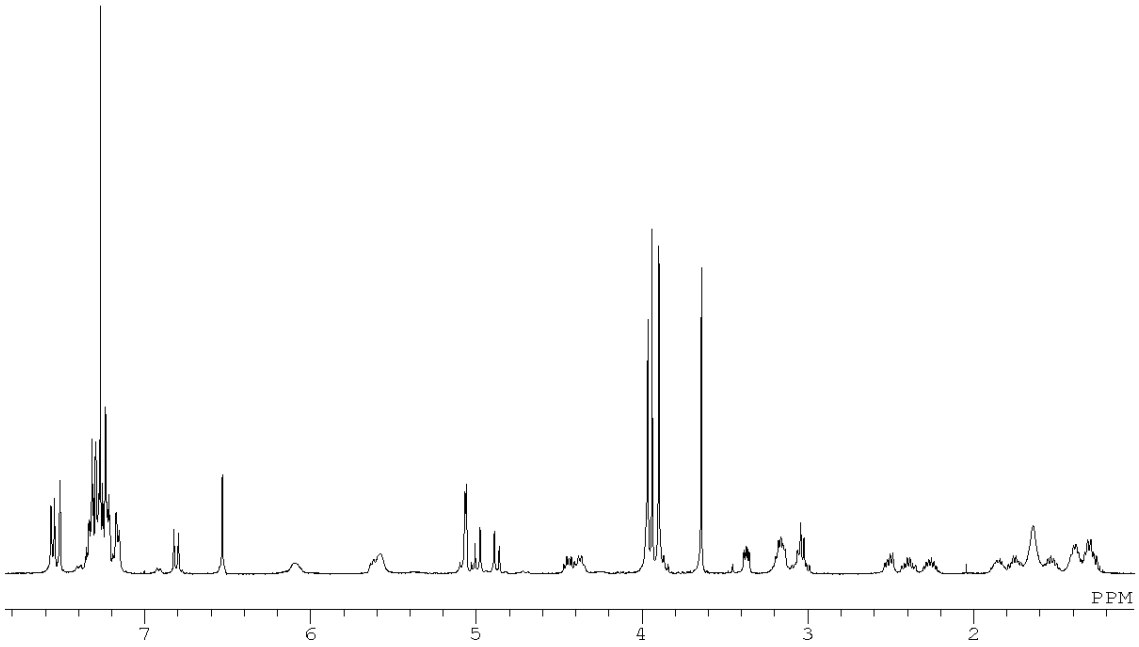


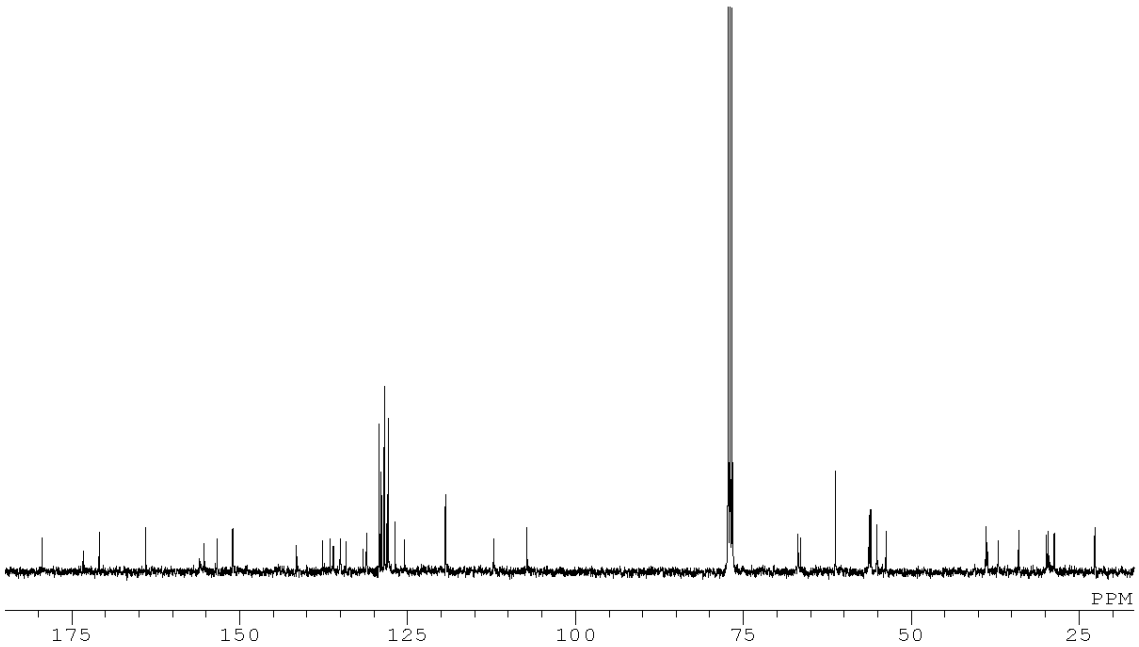


^{1}H and ^{13}C-NMR spectra for **12**

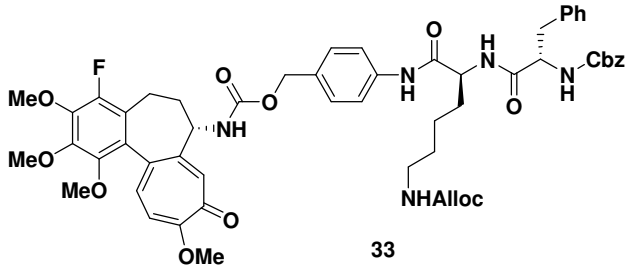


12

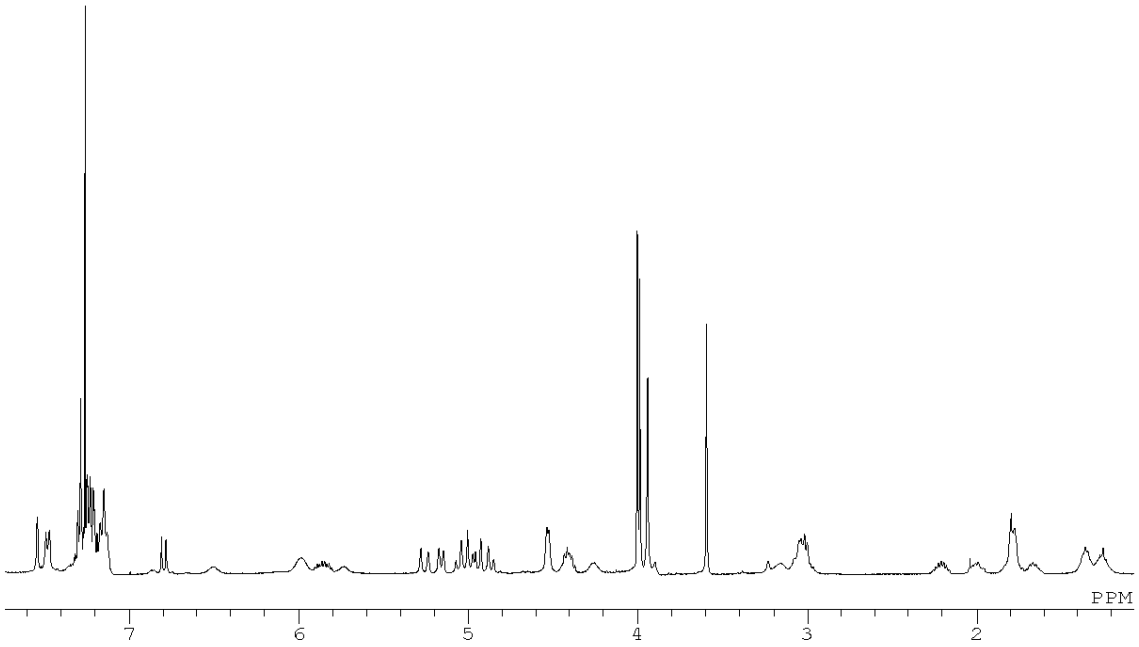


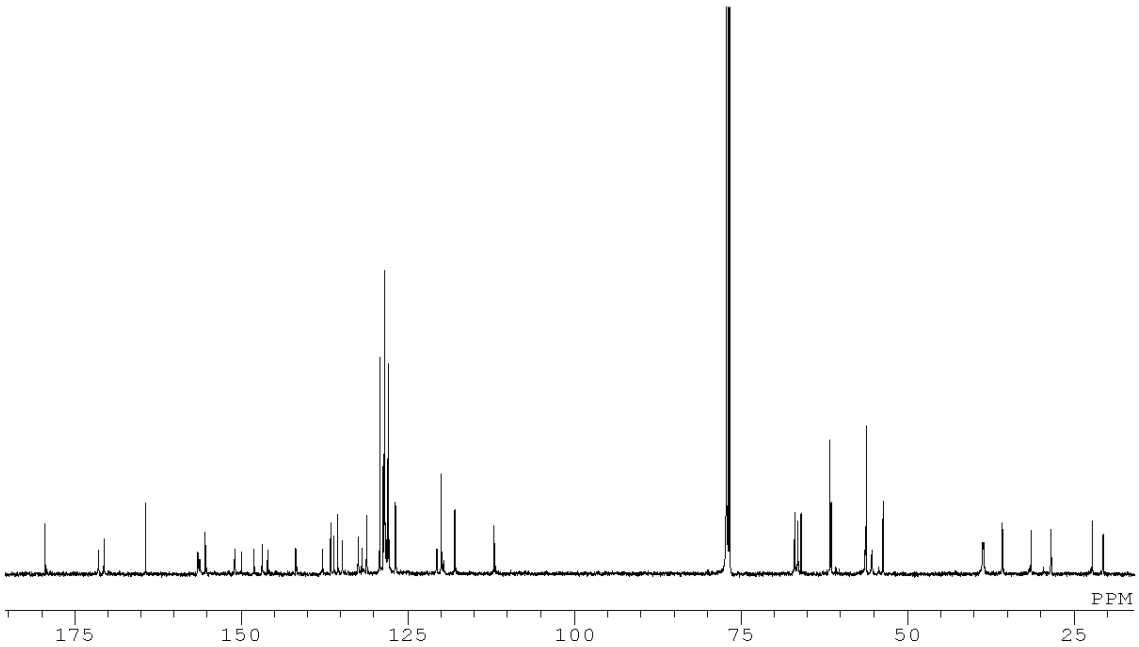


^{1}H and ^{13}C-NMR spectra for **33**

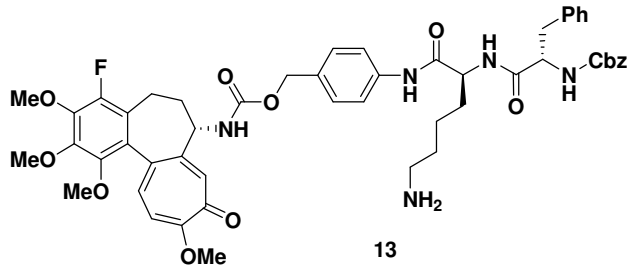


33





^{1}H and ^{13}C-NMR spectra for **13**



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