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Supporting Information

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Supporting Information

for

New, Highly Active Non-benzoquinone Geldanamycin Derivatives by Mutasynthesis

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1. Experimental

1.1 General Methods

^{1}H NMR spectra were recorded at 400 MHz with a Bruker AVS-400 or at 500 MHz with a Bruker DRX-500. ^{13}C NMR spectra were recorded at 100 MHz with a Bruker AVS-400 and at 125 MHz with a Bruker DRX-500. Chemical shift values of ^{1}H and ^{13}C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard. The multiplicities refer to the resonances in the off-resonance decoupled spectra and were elucidated using the distortionless enhancement by polarisation transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), q= quartet (methyl), t = triplet (methylene). Mass spectra (EI) were obtained at 70 eV with a type VG Autospec spectrometer (Micromass), with a type LCT (ESI) (Micromass) or with a type Q-TOF (Micromass) spectrometer in combination with a Waters Aquity Ultraperformance LC system. Analytical thin-layer chromatography was performed using precoated silica gel 60 F_{254} plates (Merck, Darmstadt), and the spots were visualized with UV light at 254 nm or by staining with H_2SO_4/4-methoxybenzaldehyde in ethanol. Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh). Sephadex chromatography was performed with LH-20 stationary phase and methanol as eluent. Isolation of geldanamycin derivatives was commonly achieved by preparative high performance liquid chromatography (Merck Hitachi, pump L-7150, interface D-7000, diode array detector L-7450).

1.2 Preparation of 3-aminobenzoic acid derivatives

The preparation of the benzoic acids were achieved according to literature procedures: **3** (ref. [S1]), **15** (ref. [S8]), **19** (ref. [S9]), **20** (ref. [S2], [S3]), **23** (ref. [S4]). Aminobenzoic acids **6**, **11**, **13**, **16** and **28** are commercially available.

*3-Amino-5-methoxymethylbenzoic acid hydrochloride (**7**)*: Borane-THF-complex (1 M, 100 mL, 100 mmol, 2 equiv) was added through a dropping funnel to a solution of 5-ni-troisophthalic acid monomethyl ester (11.2 g, 50 mmol, 1 equiv) at -10°C. The result-ing solution was heated at 90°C for 15 min. After cooling the reaction was hydrolyzed by addition of acetic acid and water (3.3 mL, 1:1 *v/v*). After neutralization (sodium bi-carbonate) THF was removed and the residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried and concentrated. Column chromatography yields 3-hydroxymethyl-5-nitro-benzoic acid methyl ester (7.6 g, 35.9 mmol, 73%) as yellow solid.

To a solution of the hydroxyester (200 mg, 0.8 mmol, 1 equiv) in ether (5 ml), pyridine (1 drop) and PBr_3 (30 µl, 0.3 mmol, 0.4 equiv) were added and stirred for 30 min. The solu-tion was diluted with ether (3 mL) and water (3 mL) was added. After 10 min the pha-ses were separated and the aqueous layer was extracted with ether three times. The combined organic extracts were dried and concentrated. Flash chromatography yields 3-bromomethyl-5-nitro-benzoic acid methyl ester (211 mg, 0.83 mmol, 88%) as light yellow solid.

A solution of the bromomethyl-benzoic acid methyl ester (500 mg, 1.8 mmol, 1 equiv) in methanol (20 mL) was treated with LiOH (1M, 9 mL, 9 mmol, 5 equiv) for 3 h at 40°C. The solution was adjusted to pH 5 (dil. HCl) and extracted with ethyl acetate. The organic extracts were dried and concentrated to yield 3-methoxymethyl-5-nitro-benzoic acid (381 mg, 1.8 mmol, 99%) as light yellow foam.

A solution of the acid (0.38 g, 1.8 mmol, 1 equiv) in ethyl acetate (20 mL) was treated with $SnCl_2*2H_2O$ (2 g, 8.9 mmol, 5 equiv) and heated at 90°C for 1 h. The mixture was poured onto ice and adjusted to pH 5-6. The aqueous layer was extracted with ethyl acetate. The combined organics were extracted with 6M hydrochloric acid. Up-on concentration with hydrochloric acid 3-amino-5-methoxymethyl benzoic acid hydrochloride **7** (0.37 g, 1.7 mmol, 96%) crystallized as colorless crystals. ^{1}H NMR (400 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm): δ 8.07 (s, 1H), 7.95 (s, 1H), 7.61 (s, 1H), 6.90 (m, 1H), 4.60 (s, 2H), 3.45 (s, 3H) ppm; ^{13}C-NMR (100 MHz, [D_4]methanol,

[D_4]methanol = 49.00 ppm): δ 167.8 (s), 143.4 (s), 134.3 (s), 132.8 (s), 129.7 (d), 126.8 (d), 124.1 (d), 74.0 (t), 58.9 ppm (q); HRMS [ESI] *m/z* for $C_9H_{10}NO_3$ [*M*-H]$^-$: calcd 180.0661, found 180.0655; mp.: 202℃ (dec.).

*5-Aminovanillinic acid hydrochloride (**8**)*: 5-Nitrovanillinic acid methyl ester was prepared from vanillinic acid following a known procedure. The spectroscopic data were in agreement with the reported data.[S5]

A solution of 5-nitrovanillinic acid methyl ester (3.6 g, 16 mmol, 1 equiv) in methanol (150 mL) was treated with LiOH (1 M, 80 mL, 80 mmol, 5 equiv) for 3 h at 40 ℃. The resulting solution was acidified with dilute hydrochloric acid and the precipitate was filtered, washed with water and dried in vacuo to obtain 5-nitro-vanillinic acid (3.4 g, 15.8 mmol, 99%) as a colorless solid.

The acid was dissolved in methanol (150 mL), treated with Pd/C (10%, 840 mg, 0.79 mmol, 0.05 equiv). The suspension was stirred under hydrogen atmosphere until complete transformation (judged by TLC). The catalyst was removed by filtration through Celite and the filtrate was concentrated in vacuo. Recrystallization from 6 M hydrochloric acid yielded 5-aminovanillinic acid hydrochloride **8** (1.7 g 7.9 mmol, 49%) as colorless solid.

^{1}H NMR (400 MHz, [D_4]methanol, [D_4]methanol = 3.31 ppm): δ 7.66 (s, 2H), 3.97 ppm (s, 3H); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.00 ppm): δ 168.4 (s), 149.5 (s), 146.4 (s), 123.3 (s), 119.3 (s), 118.8 (d), 113.7 (d), 56.9 ppm (q); HRMS [ESI] *m/z* for $C_8H_8NO_4$ [*M*-H]$^-$: calcd 182.0453, found 182.0454; m.p. 215 - 257 ℃ (dec.).

*3-Amino-5-hydroxy-4-methoxybenzoic acid hydrochloride (**9**)*: A solution of 5-nitro-vanillinic acid methyl ester (6.9 g, 30 mmol, 1 equiv) in CH_2Cl_2 (12 mL) was added dropwise to boron tribromide (1 M in CH_2Cl_2, 30 mL, 30 mmol, 1 equiv) at -78℃. The solution was warmed up to RT, stirred for 48 h and hydrolyzed with crushed ice. The organic layer was separated, the aqueous layer was extracted twice with ether. The combined organic extracts were washed with dilute NaOH and after acidification (conc. HCl) the aqueous phase was extracted three times with ether. The combined organic extracts were dried and concentrated to yield 3,4-dihydroxy-5-nitrobenzoic acid (4.6 g, 23 mmol, 75%) as a yellow solid.

The acid was dissolved in methanol (100 mL) treated with conc. sulfuric acid (2 mL) and heated under refluxing conditions for 48h. After cooling the solution was concentrated and the residue was taken up in ether, washed with brine and dried. Concentration furnished 3,4-dihydroxy-5-nitrobenzoic acid methyl ester (4.7 g, 22 mmol, 95%) as yellow solid.

To a solution of the resulting ester in pyridine (35 mL) was added acetic anhydride (2.8 g, 22 mmol, 1 equiv) over 3 h and the solution was stirred over night. After concentration the residue was taken up in water adjusted to pH 8 with sodium bicarbonate solution and extracted with ethyl acetate. The aqueous layer was acidified to pH 4 (dil. HCl) and twice extracted with ethyl acetate. The organics were dried and concentrated. Recrystallization (chloroform) yielded 3-acetoxy-4-hydroxy-5-nitrobenzoic acid methyl ester (3.4 g, 13 mmol, 60%).

The ester was dissolved in acetone (35 mL), K_2CO_3 (2.8 g, 20 mmol, 1.5 equiv) and dimethyl sulfate (2.5 g, 20 mmol, 1.5 equiv) were added and the resulting solution was heated under refluxing conditions until full transformation (judged by TLC). After cooling, water was added and the mixture was extracted with ethyl acetate (three times). The combined organic extracts were dried and concentrated to yield 3-acetoxy-4 methoxy-5-nitrobenzoic acid (3.6 g, 13 mmol, quant.) as a brown solid.

A solution of the acetate in methanol (40 mL) was treated with sodium methylate (2.5 M, 6.2 mL, 15.5 mol, 1.15 equiv) for 3 h. The solution was concentrated and the residue was taken up in dil. sulfuric acid and neutralized with sodium bicarbonate solution, extracted with ethyl acetate, dried and concentrated to yield 3-hydroxy-4-methoxy-5-nitrobenzoic acid methyl ester (2.1 g, 9.4 mmol, 70%).

A solution of the ester in methanol (90 mL) was treated with LiOH (1 M, 50 mL, 50 mmol, 5 equiv) for 3 h at 40 ℃. The resulting solu tion was acidified with dilute hydrochloric acid and the precipitate was filtered off, washed with water and dried in vacuo to obtain 3-hydroxy-4-methoxy-5-nitrobenzoic acid (1.9 g, 9.1 mmol, 96%) as a colorless solid.

The acid was dissolved in methanol (40 mL) treated with Pd/C (10%, 190 mg, 0.18 mmol, 0.02 equiv). The suspension was stirred under hydrogen atmosphere until full transformation (judged by TLC). The catalyst was removed by filtration over Celite and the filtrate was concentrated. Recrystallization from 6M hydrochloric acid yielded

3-hydroxy-4-methoxy-5-aminobenzoic acid **9** (0.31 g, 1.4 mmol, 15%) as a colorless solid.

^{1}H NMR (400 MHz, [D_4]methanol, [D_4]methanol = 3.31 ppm): δ 7.60 (d, *J* = 1.7 Hz, 1H), 7.53 (d, *J* = 1.7 Hz, 1H), 4.09 ppm (s, 3H); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.00 ppm): δ 167.9 (s), 151.7 (s), 146.4 (s), 127.6 (s), 125.0 (s), 119.9 (d), 116.7 (d), 61.6 ppm (q); HRMS [ESI] *m/z* for $C_8H_8NO_4$ [*M*-H]$^-$: calcd 182.0453, found 182.0451; m.p. 226 - 258℃ (dec.).

*3-Amino-5-(trifluoromethyl)benzoic acid-hydrochloride (**14**)*: A solution of 3-nitro-5-(trifluoromethyl)benzoic acid (1 g, 4.25 mmol, 1 equiv) in methanol (35 mL) was charged with 10% Pd/C (10%; 227 mg, 0.21 mmol, 0.05 equiv) and stirred under hydrogen atmosphere for 16 h at room temperature. The catalyst was removed by centrifugation (4000 *g*, 10 min) and the supernatant was evaporated to dryness. The residue was taken up withethyl acetate (5 mL), precipitated by addition of five volumes of pentane and filtered. The procedure was repeated with the filtrate to obtain 805 mg of crude product which was recrystallised from 6 M HCl (70 mL) and dried in vacuo to yield 3-amino-5-(trifluoromethyl)benzoic acid-hydrochloride (393 mg, 1.63 mmol, 36 %) as white needles.

^{1}H NMR (400 MHz, [D_4]methanol, [D_4]methanol = 3.31 ppm): δ 8.33 (s, 1H), 8.27 (s, 1H), 7.93 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.00 ppm): 166.4 (s), 135.7 (d), 134.8 (d), 133.7 (q, $J_{C\text{-}F}$ = 33.9 Hz), 125.9 (dq, $J_{C\text{-}F}$ = 216.2, 3.8 Hz), 125.8 (s), 123.1 (s); HRMS [ESI] *m/z* for $C_8H_5NO_2F_3$ [*M*-H]$^-$: calcd 204.0272, found 204.0276; m.p. 179-208℃ (dec.).

*3-Chloromethyl-5-amino-benzoic acid hydrochloride (**7**)*: A solution of 3-hydroxymethyl-5-nitro-benzoic acid methyl ester (250 mg, 1.18 mmol, 1 equiv) in pyridine (3 mL) is cooled to -10℃ and thionyl chloride (211 mg, 1.77 mmol, 1.6 equiv) is added. The resulting mixture is stirred over night at RT. Ether is added and the organic layer is washed with dil. HCl (three times). After drying and concentration 3-chloromethyl-5-nitrobenzoic acid methyl ester (100 mg, 0.43 mmol, 37%) is obtained as colorless solid.

A solution of 3-chloromethyl-5-nitro benzoic acid methyl ester (4.9 g, 21.3 mmol, 1 equiv) in THF (100 mL) was treated with LiOH (1 M, 107 mmol , 5 equiv) for 3 h at 40℃. The solution is adjusted to pH 5 (dil. HCl) a nd extracted with ethyl acetate. The

organic extracts were dried and concentrated to yield 3-chloromethyl-5-nitro benzoic acid (4.0 mg, 18.6 mmol, 87%) as brown solid.

A solution of the nitro-benzoic acid (3 g, 14 mmol, 1 equiv) in ethyl acetate (150 mL) is treated with $SnCl_2 \bullet 2H_2O$ (9.5 g, 42 mmol, 3 equiv) and refluxed for 1 h. The reaction mixture is poured in ice water and adjusted to pH 5 (dil. HCl). The aqueous layer is extracted with ethyl acetate (three times). The combined organics are extracted with 6 M HCl. 3-chloromethyl-5-amino-benzoic acid hydrochloride (3 g, 13.5 mmol, 96%) crystallizes on concentration of the aqueous layer.

^{1}H NMR (200 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm) δ 8.17 (dd, *J* = 1.5, 2.0 Hz, 1H), 8.00 (dd, *J* = 1.5, 2.0 Hz, 1H), 7.72 (dd, *J* = 1.5, 2.0 Hz, 1H), 4.79 ppm (s, 2H); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.0 ppm) δ 167.4 (s), 142.5 (s), 134.8 (s) 132.9 (s), 131.1 (d), 128.4 (d), 124.9 (d), 45.2 (t) ppm; HRMS [ESI] *m/z* for $C_8H_7NO_2Cl$ [*M*-H]$^-$: calcd 227.9660, found 227.9662; m.p. 173-209 ℃ (dec.).

*3-Bromomethyl-5-amino-benzoic acid hydrochloride (**18**)*: A solution of 3-bromomethyl-5-nitro benzoic acid methyl ester (see preparation of **7**, 100 mg, 0.36 mmol, 1 equiv) in THF (5 mL) was treated with LiOH (1 M, 1.8 mmol , 5 equiv) for 3h at 40℃. The solution is adjusted to pH 5 (dil. HCl) and extracted with ethyl acetate. The organic extracts were dried and concentrated to yield 3-bromomethyl-5-nitro benzoic acid (89 mg, 0.34 mmol, 95%) as yellow solid.

A solution of the nitro-benzoic acid (80 mg, 0.31 mmol, 1 equiv) in ethyl acetate (5 mL) is treated with $SnCl_2$*$2H_2O$ (350 mg, 1.55 mmol, 5 equiv) and refluxed for 1 h. The reaction mixture is poured in ice water and adjusted to pH 5 (dil. HCl). The aqueous layer is extracted with ethyl acetate (three times). The combined organics are extracted with 6 M HCl. 3-bromomethyl-5-amino-benzoic acid hydrochloride (42 mg, 0.25 mmol, 81%) crystallizes on concentration of the aqueous layer.

^{1}H NMR (400 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm) δ 8.02 (s, 1H), 8.00 (s, 1H), 7.76 (s, 1H), 4.79 ppm (s, 2H); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.0 ppm) δ 167.4 (s), 142.4 (s), 134.7 (s), 132.8 (s), 131.1 (d), 128.5 (d), 125.0 (d) 45.2 (t) ppm; HRMS [ESI] *m/z* for $C_8H_7NO_2Br$ [*M*-H]$^-$: calcd 227.9660, found 227.9652; m.p. 180 ℃ (dec.).

*3-Amino-5-bromo-benzoic acid (**25**)*: Aminobenzoic acid **25** was obtained in two steps from 3-nitrobenzoic acid. 3-Nitrobenzoic acid (4.26 g, 25.5 mmol, 1 equiv) and silver

nitrate (4.21 g, 13.5 mmol, 0.53 equiv) were dissolved in conc. sulfuric acid (5 mL). Bromine (1.5 mL, 29.3 mmol, 1.15 equiv) was slowly added with vigorous stirring and the resulting mixture was heated under refluxing conditions for 4 h. After cooling the mixture was poured in ice-water and the resulting solid was removed by filtration. The crude acid was dissolved in sodium bicarbonate solution and recrystallized after addition of dilute hydrochloric acid (5.46 g, 22.2 mmol, 87%). 3-Bromo-5-nitro-benzoic acid (5.13 g, 20 mmol, 1 equiv) and stannous chloride (23.89 g, 106 mmol, 5.3 equiv) were dissolved in 100 mL glacial acetic acid and heated under refluxing conditions for 30 min. The reaction solution was poured onto ice and adjusted to pH 7-8 by addition of sodium bicarbonate solution. The resulting solution was heated and then filtered. The filtrate was acidified (pH 6) and the precipitated 3-bromo-5-amino-benzoic acid **25** was filtered and recrystallized from ethanol (3.59 g, 16.6 mmol, 83%).

^{1}H NMR (400 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm) δ 7.34 (1H, dd, *J* = 1.5, 1.5 Hz), 7.26 (1H, dd, *J* = 1.5, 1.5 Hz), 7.02 ppm (1H, dd, *J* = 1.5, 1.5 Hz); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.0 ppm) δ 169.0 (s), 151.3 (s), 134.3 (s), 123.6 (s), 122.1 (d), 121.6 (d), 115.6 (d) ppm; HR-MS [ESI] *m/z* calcd for $C_7H_5NO_2Br$ [*M*-H^-] 213.9504, found 213.9514; m.p. 192-207℃.

Preparation of aminobenzoic acids 4, 10 and 12

General procedure for the alkylation of 3*-tert-*butoxycarbonylamino-5-hydroxy-benzoic acid methyl ester: A solution of 3-*tert*-butoxycarbonylamino-5-hydroxy-benzoic acid methyl ester in acetone (0.2 M) is treated with K_2CO_3 (2 equiv) and alkyl iodide (3 equiv). The resulting mixture is stirred at 40℃ for 24 h and concentrated under reduced pressure. The residue is purified by flash chromatography (for ethyl ether and allyl ether) or crystallization (for trideutero methyl ether).

3-tert-*Butoxycarbonylamino-5-ethoxy-benzoic acid methyl ester* (450 mg, 1.53 mmol, 76%)

3-Allyloxy-5-tert-*butoxycarbonylamino-benzoic acid methyl ester* (550 mg, 1.8 mmol, 90%)

3-tert-*Butoxycarbonylamino-5-trideuteromethoxy-benzoic acid methyl ester* (545 mg, 1.92 mmol, 85%).

General procedure for the saponification of alkoxy-tert-butoxycarbonylamino-benzoic acid methyl esters: A solution of the methyl ester in methanol (0.2 M) is treated with LiOH (1 M, 5 equiv) for 3 h at 45 °C. The resulting solution is acidified with dilute hydrochloric acid and the precipitate filtered off, washed with water and dried in vacuo to obtain the benzoic acid.

3-tert-*Butoxycarbonylamino-5-ethoxy-benzoic acid* (383 mg, 1.36 mmol, 95%)

3-Allyloxy-5-tert-*butoxycarbonylamino-benzoic acid* (490 mg, 1.67 mmol, 99%)

3-tert-*Butoxycarbonylamino-5-trideuteromethoxy-benzoic acid* (428 mg, 1.58 mmol, 93%).

General procedure for the carbamate removal of Boc-protected aminobenzoic acids: A solution of the carbamate in CH_2Cl_2 (0.5 M) is treated with TFA (40 equiv) and stirred for 24 h. The solution is neutralized with sodium bicarbonate solution and the layers are separated. The aqueous layer is adjusted to pH 5 (dil. HCl) and extracted with ethyl acetate (thrice). The combined organics are dried and concentrated to yield the aminobenzoic acid.

3-Amino-5-ethoxybenzoic acid (210 mg, 1.16 mmol, 94%). This material was dissolved in dioxane (0.5 M) and treated with HCl (4 M in dioxane, 1.7 equiv). After removal of the solvent 3-amino-5-ethoxybenzoic acid hydrochloride **10** was obtained in quantitative yield.

^{1}H NMR (400 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm): δ 7.53 (m, 2H), 7.09 (m, 1H), 4.13 (q, *J* = 7.0 Hz, 2H), 1.43 ppm (t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.00 ppm): δ 168.1 (s), 161.6 (s), 153.3 (s), 135.1 (s), 116.2 (d), 115.4 (d), 114.0 (d), 65.4 (t), 14.9 ppm (q); HRMS [ESI] *m/z* for $C_9H_{12}NO_3Cl$ [*M*-HCl-H]$^-$: calcd 180.0661, found 180.0659; m.p. 200 - 230°C (dec.).

*3-Allyloxy-5-aminobenzoic acid (**4**)* (120 mg, 0.62 mmol, 87%): ^{1}H NMR (400 MHz, [D_6]DMSO, [D_5]DMSO = 2.50 ppm): δ 12.62 (br s, 1H), 6.80 (dd, *J* = 1.9, 1.4 Hz, 1H), 6.63 (dd, *J* = 2.4, 1.4 Hz, 1H), 6.35 (dd, *J* = 2.4, 1.9 Hz, 1H), 6.02 (ddt, *J* = 17.3, 10.5, 5.2 Hz, 1H), 5.36 (ddt, *J* = 17.3, 1.6, 1.6 Hz, 1H), 5.32 (br s, 2H), 5.24 (ddt, *J* = 10.5, 1.6, 1.6 Hz, 1H), 4.49 ppm (ddd, *J* = 5.2, 1.6, 1.6 Hz, 2H); ^{13}C NMR (100 MHz, [D_6]-DMSO, [D_6]DMSO = 39.52 ppm): δ 167.8 (s), 159.0 (s), 150.1 (s), 133.8 (d), 132.3 (s), 117.1 (t), 108.2 (d), 104.2 (d), 102.5 (d), 67.9 ppm (t); HRMS [ESI] *m/z* for $C_{10}H_{11}NO_3$ [*M*-H]$^-$: calcd 192.0661, found 192.0664; m.p. 132°C.

*3-Amino-trideuteromethoxybenzoic acid (**12**)* (170 mg, 1.0 mmol, 67%): ^{1}H NMR (400 MHz, [D_4]methanol, [D_3]methanol = 3.31 ppm): δ 7.37 (m, 1H), 7.35 (m, 1H), 6.90 (m, 1H), 4.13 (q, *J* = 7.0 Hz, 2H)1.43 ppm (t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, [D_4]methanol, [D_4]methanol = 49.00 ppm): δ 169.0 (s), 162.2 (s), 141.9 (s), 134.5 (s), 113.8 (d), 110.7 (d), 110.5 (d), 55.4 ppm (sep $J_{C\text{-}D}$ = 22 Hz); HRMS [ESI] *m/z* for $C_8H_6D_3NO_3$ [*M*-H]$^-$: calcd 169.0692, found 169.0691; m.p. 170℃ (dec.) .

1.3 Feeding experiments

General parameters: *Streptomyces hygroscopicus* mutant K390-61-1 was stored as stock cultures at 4℃ on R5-agar plates in the refr igerator. The strain was grown on a R5 agar plate at 30℃ for 7 days. A single colony o f this 7 days old agar plate of K390-61-1 mutant was used to inoculate the GYP precultures which were incubated at 28℃ for 2 days. Precultures were prepared in GY P-medium (40 mL/flask, 2.5 g/L yeast extract, 10 g/L peptone, 10 g/L glucose, 3 g/L xanthan gum, distilled water). Main cultures were prepared in GP-medium (40 g/L glucose, 2.5 g/L peptone, 2.5 g/L tryptone, 5 g/L oatmeal, 2.5 g/L yeast extract, 3 g/L xanthan gum, distilled water). Liquid culture fermentations were incubated at 30 ℃ with vigorous shaking (200 rpm) in 500 mL Erlenmeyer flasks with a baffle. Main cultures were inoculated with 1 mL preculture per 25 mL culture broth. The production cultures were harvested after 7 d of fermentation and extracted twice with EtOAc. The EtOAc extracts were concentrated, and the residue was dissolved in MeOH (1 ml) and used directly for ESI-MS analysis.

Cultivation parameters

Feeding experiments (small scale): Benzoic acid derivatives **4 - 19** (37.5 μmol, as 25 mM sterile solution in DMSO/H_2O 1:1) were added in four 250 μL portions at 48, 72, 96 and 120 h after inoculation to a 25 mL main culture of *S. hygroscopicus* mutant K390-61-1.

Geldanamycin derivatives expected to be formed from supplementing aminobenzoic acids **14** – **19** could not be detected after UPLC-ESI-HRMS analysis of the extracts. After feeding precursors **4** - **13** and **20**, **23**, **25**, and **28** employing the fermentation conditions described above, UPLC-ESI-HRMS analysis revealed for all fermentations product [*M*+H]$^+$ peaks. Commonly, (except for aminobenzoic acids **6** and **11**), the amount of new geldanamycin derivatives generated was too low for preparative isola-

tion. Scale-up was not carried out for mutasynthons **6** and **11** because the products have been described before.[S6, S7]

Feeding experiments (large scale): **A:** Benzoic acid derivatives **3, 20, 23, 25** and **28** (37.5 µmol, as 25 mM sterile solution in DMSO/H_2O= 1:1 or pure DMSO (compound **28**)) were added in four 250 µL portions at 48, 72, 96 and 120 h after inoculation to a 25 mL main culture of *S. hygroscopicus* mutant K390-61-1.

B1: For scale-up fermentation **20** (300 mg, 1.8 mmol, as 70 mM sterile solution in DMSO/H_2O 1:1) was fed in four equal portions at 48, 72, 96 and 120 h after inoculation to 14 70 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded 17-demethoxy-18-*O*-methyl reblastatin (**21**; 7 mg, 1.3 µmol) and 17-desmethyl-18-*O*-methyl-4,5-dehydro reblastatin (**22**; 2.4 mg, 0.4 µmol) after silica gel filtration, Sephadex LH-20 chromatography and two HPLC purification steps. HPLC conditions employed for the first step were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18 AQ, 30x20 mm, 10 µm, endc.; c) solvents: methanol/H_2O and d) program: from 20 to 100% methanol within 100 min, 20 min 100 % methanol at 5 mL/min flow rate. For the second step they were a) column: Reprosil-Pur 120 C18 AQ, 250 x 8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18 AQ, 40x8 mm, 5 µm, endc.; c) solvents: acetonitrile/H_2O and d) program: from 10 to 50% acetonitrile within 50 min, from 50 to 70% acetonitrile within 5 min, 5 min 70% acetonitrile at 2.5 mL/min flow rate.

B2: For scale-up fermentation of **23** (415 mg, 2.7 mmol, as 70 mM sterile solution in DMSO/H_2O 1:1) was fed in four equal portions at 48, 72, 96 and 120 h after inoculation to 14 70 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded 17-demethoxy-18-dehydroxy-19-fluoro reblastatin (**24**; 1.2 mg, 7.7 µmol) after silica gel filtration, Sephadex LH-20 chromatography and HPLC purification. HPLC conditions employed were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc. ; b) guard: Reprosil-Pur 120 C18 AQ, 30 x 20 mm, 10 µm, endc.; c) solvents: methanol / H_2O; d) program: from 20% to 100% methanol within 100 min, 20 min 100% methanol at 5 mL/min flow rate.

B3: For scale-up fermentation of **25** (580 mg, 2.7 mmol, as 70 mM sterile solution in DMSO/H_2O 1:1) was fed in four equal portions at 48, 72, 96 and 120 h after inoculation to 14 70 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded 18-bromo-17-demethoxyreblastatin (**26**; 0.9 mg, 0.16 µmol) and 18-

bromo-17-demethyl reblastatin (**27**; 0.9 mg, 0.15 µmol) after silica gel filtration, Sephadex LH-20 chromatography and HPLC purification. HPLC conditions employed were a) column: Reprosil-Pur 120 C18 AQ, 250x25 mm, 5 µm, endc. ; b) guard: Reprosil-Pur 120 C18 AQ, 30 x 20 mm, 10 µm, endc. c) solvents: methanol / H_2O; d) program: from 20% to 100% methanol within 100 min, 20 min 100% methanol at 5 mL/min low rate.

B4: For scale-up fermentation of **28** (250 mg, 1.8 mmol, as 70 mM sterile solution in DMSO) was fed in four equal portions at 48, 72, 96 and 120 h after inoculation to 14 70 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded 18-aza-reblastatin (**29**; 1.4 mg, 0.3 µmol) after silica gel filtration, Sephadex LH-20 chromatography and HPLC purification. HPLC conditions employed were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc. ; b) guard: Reprosil-Pur 120 C18 AQ, 30 x 20 mm, 10 µm, endc.; c) solvents: methanol / H_2O; d) program: from 20 to 100% methanol within 100 min, 20 min 100% methanol at 5 mL/min flow rate.

1.4 Spectroscopic data of mutaproducts

*17-Demethoxy-18-O-methyl reblastatin (**21**)*: ^{1}H NMR (500 MHz, *T* = 320 K, $CDCl_3$, $CHCl_3$ = 7.26 ppm): *δ* 7.52 (s, 1H, NH), 6.88 (s, 1H, 17-H), 6.47 (s, 1H, 19-H), 6.40 (s, 1H, 21-H), 5.87 (t, 1H, *J* = 6.6 Hz, 3-H), 5.30 (d, 1H, *J* = 10.1 Hz, 9-H), 5.10 (d, 1H, *J* = 5.4 Hz, 7-H), 4.62 (s, 2H, NH_2), 3.78 (s, 3H, 20-OMe), 3.65-3.62 (m, 1H, 11-H), 3.40 (s, 3H, 12-OMe), 3.35 (s, 3H, 6-OMe), 3.27 (m, 1H, 6-H), 3.11-3.09 (m, 1H, 12-H), 2.70 (dd, 1H, *J* = 13.2, 4.6 Hz, 15-H_a), 2:46-2.43 (m, 1H, 10-H), 2.37 (dd, 1H, *J* = 13.2, 6.1 Hz, 15-H_b), 2.40-2.30 (m, 1H, 4-H_a), 2.21-2.11 (m, 1H, 4-H_b), 2.04 (s, 1H, 11-OH) 1.96-1.90 (m, 1H, 14-H), 1.87 (s, 3H, 2-Me), 1.57-1.52 (m, 1H, 13-H_a) 1.46 (s, 3H, 8-Me), 1.43-1.38 (m, 1H, 5-H_a), 1.35-1.30 (m, 1H, 5-H_b), 1.25-1.20 (m, 1H, 13-H_b), 1.07 (d, 3H, *J* = 6.5 Hz, 10-Me), 0.80 ppm (d, 3H, *J* = 6.5 Hz, 14-Me). ^{13}C NMR (125 MHz, *T* = 320K, $CDCl_3$, $CDCl_3$ = 77.2 ppm): *δ* 160.1 (s, C-1), 155.9 (s, $COONH_2$), 152.8 (s, C-20), 142.2 (s, C-19), 139.97 (s, C-16), 134.4 (d, C-3), 132.3 (s, C-2), 131.6 (d, C-9), 130.3 (s, C-8), 116.6 (d, C-21), 112.8 (d, C-19), 104.7 (d, C-17), 81.3 (d, C-7), 81.1 (d, C-12), 80.0 (d, C-6), 73.8 (d, C-11), 58.8 (q, C-12 OMe), 56.7 (q, C-6 OMe), 55.3 (q, C-20 OMe), 42.9 (t, C-15), 34.3 (d, C-10), 32.8 (d, C-13), 31.2 (d, C-14), 29.3 (d, C-5), 24.1 (d, C-4), 18.6 (q, C-14 Me), 17.3 (q, C-10 Me), 13.4 (q, C-2

Me), 12.4 ppm (q, C-8 Me). HRMS [ESI] *m/z* for $C_{29}H_{44}N_2O_7Na$ [*M*+Na]$^+$: calcd 555.3046, found 555.3049.

*17-Demethyl-18-O-methyl-4,5-dehydro reblastatin (**22**)*: ^{1}H NMR (500 MHz, T = 295K, CD_2Cl_2, $CHDCl_2$ = 5.32 ppm): *δ* 8.51 (br. s, 1H, NH), 7.80 (br. s, 1H, 19-H), 7.03 (br. s, 1H, 3-H), 6.51 (t, 1H, *J* = 10.0 Hz, 4-H) 6.33 (d, 1H, *J* = 3.0 Hz, 21-H), 5.79 (d, 1H, *J* = 10.0 Hz, 9-H), 5.68 (d, 1H, *J* = 10.0 Hz, 5-H), 5.01 (ps. s, 1H, 7-H), 4.71 (br. s, 2H, NH_2), 4.31 (d, 1H, *J* = 10.0 Hz, 6-H), 3.74 (s, 3H, 12-OMe), 3.60-3.57 (m, 1H, 11-H), 3.41 (s, 3H, 18-OMe), 3.41 (ps. s, 1H, 12-H) 3.19 (s, 3H, 6-OMe), 2.82 (t, 1H, *J* = 6.6 Hz, 10-H), 2.72 (d, 1H, *J* = 12.5 Hz, 15-H_a), 2.51-2.33 (m, 1H, 15-H_b), 1.86 (s, 3H, 2-Me), 1.83-1.79 (m, 1H, 14-H), 1.75 (s, 3H, 8-Me), 1.63-1.57 (m, 1H, 13-H_a), 1.33-1.30 (m, 1H, 13-H_b), 0.98 (d, 3H, *J* = 6.5 Hz, 14-Me), 0.93 ppm (d, 3H, *J* = 7.2 Hz, 10-Me). ^{13}C NMR (125 MHz, *T* = 295 K, CD_2Cl_2, CD_2Cl_2 = 53.5 ppm): *δ* 156.1 (s, $COONH_2$), 152.5 (s, C-1), 146.5 (s, C-18), 138.4 (s, C-2), 137.4 (s, C-17), 132.8 (d, C-5), 131.8 (d, C-9), 129.9 (s, C-8), 128.1 (s, C-arom), 127.4 (s, C-arom), 127.2 (d, C-4), 126.0 (d, C-3), 111.0 (d, C-21), 102.6 (d, C-19), 83.0 (d, C-12), 81.7 (d, C-7), 80.7 (d, C-6), 74.5 (d, C-11), 57.5 (q, C-12 OMe), 56.6 (q, C-18 OMe), 55.6 (q, C-6 OMe), 40.5 (t, C-15), 37.5 (d, C-14), 32.6 (d, C-10), 29.7 (d, C-13), 22.7 (q, C-14 Me), 13.9 (q, C-10 Me), 12.7 (q, C-2 Me), 12.3 ppm (q, C-8 Me). HRMS [ESI] *m/z* for $C_{29}H_{42}N_2O_8Na$ [*M*+Na]$^+$: calcd 569.2839, found: 569.2839.

Selected HMBC-correlations for **22**

δ-H [ppm]	#	HMBC
8.51	N-H	C19 (102.6, d); C21 (111.0, d)
2.72	15H_a	-*
2.51-2.33	15H_b	-*
3.41	18-OMe	-*

* low resolution due to broad signals.

*17-Demethoxy-18-dehydroxy-19-fluoro reblastatin (**24**)*: ^{1}H NMR (500 MHz, *T* = 295K, $CDCl_3$, $CHCl_3$ = 7.26 ppm): *δ* 7.80 (br.s, 1H, NH), 7.24-7.20 (m, 1H, 21-H) 6.93 (ps. t, 1H, $J_{H\text{-}H}$ = 8.0 Hz, 18-H), 6.83 (dd, 1H, $J_{F\text{-}H}$ = 6.3 Hz, $J_{H\text{-}H}$ = 2.7 Hz, 17-H), 5.85 (td, 1H, *J* = 6.4, 0.5 Hz, 3-H), 5.30 (d, 1H, *J* = 9.8 Hz, 9-H), 5.11 (d, 1H, *J* = 5.4 Hz, 7-H), 4.84 (br. s, 2H, NH_2), 3.63 (dd, 1H, *J* = 8.5, 3.5 Hz, 11-H), 3.38 (s, 3H, 12-OMe), 3.34 (s, 3H, 6-OMe), 3.27 (dt, 1H, *J* = 7.4, 5.4 Hz, 6-H), 3.12 (dt, 1H, *J* = 8.5, 3.5 Hz, 12-H), 2.72 (dd, 1H, *J* = 13.6, 6.0 Hz, 15-H_a) 2.52 (ddd, 1H, *J* = 13.6, 4.8 Hz, 15-H_b), 2.48-

2.42 (m, 1H, 10-H), 2.38-2.29 (m, 1H, 4-H_a), 2.21-2.12 (m, 1H, 4-H_b), 2.10-2.03 (m, 1H, 5-H_a), 1.93 (m, 1H, 14-H), 1.84 (s, 3H, 2-Me), 1.68-1.61 (m, 1H, 13-H_a), 1.50 (s, 3H, 8-Me), 1.48-1.41 (m, 1-H, 5-H_b), 1.24-1.14 (m, 1H, 13-H_b), 1.07 (d, 3H, *J* = 6.5 Hz 10-Me) 0.83 ppm (d, 3H, *J* = 6.8 Hz 14-Me); ^{13}C NMR (125 MHz, *T* = 295K, $CDCl_3$, $CDCl_3$ = 77.2 ppm): *δ* 171.8 (s, C-1), 158.9 (d, J_{F-C} = 243.6 Hz, C-19), 156.3 (s, $COONH_2$), 138.9 (s, C-16), 135.0 (s, C-2), 134.7 (d, C-3), 131.8 (d, C-9), 130.4 (s, C-8), 128.9 (d, C-17), 128.0 (s, J_{F-C} = 18.0 Hz, C-20), 121.4 (d, J_{F-C} = 8.4 Hz, C-21), 115.8 (d, J_{F-C} = 24.5 Hz, C-18), 81.3 (d, C-7), 81.1 (d, C-12) 80.4 (d, C-6), 74.1 (d,. C-11), 58,9 (q, C-12 OMe), 56.9 (q, C-6 OMe), 35.1 (t, C-15), 34.5 (d, C-10), 33.1 (t, C-13), 31.4 (d, C-14), 29.6 (t, C-5), 24.5 (t, C-4), 18.6 (q, C-14 Me), 17.5 (q, C-10 Me), 13.6 (q, C-2 Me), 12.6 ppm (q, C-8 Me). HRMS [ESI] *m/z* for $C_{28}H_{41}N_2O_6FNa$ [*M*+Na]$^+$: calcd 543.2846, found 543.2844.

*18-Bromo-17-demethoxy reblastatin (**26**)*: ^{1}H NMR (500 MHz, *T* = 295K, [D_4]methanol, [D_3]methanol = 3.31 ppm): *δ* 7.35 (br. s, 1H, 19-H), 7.10 (s, 1H, 17-H), 6.79 (s, 1H, 21-H), 5.76 (br. s, 1H, 3-H), 5.26 (d, 1H, *J* = 9.8 Hz, 9-H), 4.87 (underneath H_2O, 1H, 7-H), 3.55 (m, 1H, 11-H), 3.44 (s, 3H, 12-OMe), 3.35 (s, 3H, 6-OMe), 3.31 (underneath MeOH, 1H, 6-H), 3.10 (dt, 1H, *J* = 10.0, 3.0 Hz, 12-H), 2.72 (dd, 1H, *J* = 13.2, 5.0 Hz, 15-H_a), 2.52 (dd, 1H, *J* = 13.2, 5.0 Hz, 15-H_b), 2.43-2.38 (m, 1H, 10-H), 2.35-2.28 (m, 1H, 4-H_a), 2.16-2.07 (m, 1H, 4-H_b), 2.04-1.95 (m, 1H, 14-H), 1.85 (s, 3H, 2-Me), 1.66-1.59 (m, 2H, 13-H), 1.45 (s, 3H, 8-Me), 1.23-1.15 (m, 2H, 5-H), 1.03 (d, 3H, *J* = 6.6 Hz, 10-Me) 0.79 ppm (d, 3H, *J* = 6.6 Hz, 14-Me); ^{13}C NMR (125 MHz, *T* = 295K, [D_4]methanol, [D_4]methanol = 49.0 ppm): *δ* 174.7 (s, C-1), 159.1 (s, $COONH_2$), 143.9 (s, C-20), 141.6 (s, C-16), 136.9 (d, C-3), 134.2 (d, C-9), 131.9 (s, C-2), 131.5 (s, C-8), 130.8 (d, C-17), 125.6 (d, C-21), 123.4 (d, C-19), 123.0 (s, C-18), 83.4 (d, C-7), 81.7 (d, C-12), 81.0 (d, C-6), 75.0 (d, C-11), 59.8 (q, C-12 OMe), 57.2 (q, C-6 OMe), 43.4 (t, C-15), 36.0 (d, C-10), 31.2 (t, C-13), 30.5 (d, C-5), 28.1 (t, C-14), 24.5 (t, C-4), 18.4 (q, C-14 Me), 17.7 (q, C-10 Me), 13.7 (q, C-2 Me), 12.4 ppm (q, C-8 Me); HRMS [ESI] *m/z* for $C_{28}H_{41}N_2O_6BrNa$ [*M*+Na]$^+$: calcd 603.2046, found 603.2047.

*18-Bromo-17-demethyl reblastatin (**27**)*: ^{1}H NMR (500 MHz, *T* = 295 K, [D_4]methanol, [D_3]methanol = 3.31 ppm): *δ* 7.26 (br. s, 1H, 19-H), 6.73 (s, 1H, 21-H), 5.74 (br. s, 1H, 3-H), 5.22 (br. s, 1H, 9-H), 4.87 (underneath H_2O, 1H, 7-H), 3.55 (br. s, 1H, 11-H), 3.44 (s, 3H, 12-OMe), 3.35 (s, 3H, 6-OMe), 3.31 (beneath MeOH, 1H, 6-H), 3.11-3.0 (m, 1H, 12-H), 2.97-2.93 (m, 1H, 15-H_a), 2.50-2.42 (m, 1H, 15-H_b), 2.50-2.42 (m, 1H, 10-H), 2.33-2.27 (m, 1H, 4-H_a), 2.16-2.10 (m, 1H, 4-H_b), 2.07-2.02 (m, 1H, 14-H),

1.82 (s, 3H, 2-Me) ~1.82-1.70 (m, 1H, 5-H_a), 1.66-1.53 (m, 1H, 5-H_b), 1.45 (s, 3H, 8-Me), 1.34-1.29 (m, 1H, 13-H_a), 1.26-1.17 (m, 1H, 13-H_b), 1.02 (d, 3H, *J* = 6.4 Hz, 10-Me) 0.81 ppm (br. s, 3H, 14-Me); ^{13}C NMR (125 MHz, *T* = 295 K, [D_4]methanol, [D_3]-methanol = 49.0 ppm): *δ* 171.7 (s, C-1), 159.1 (s, $COONH_2$), 151.2 (s, C-17), 136.5 (d, C-3), 134.5 (d, C-9), 132.9 (s, C-arom), 132.4 (s, C-2), 131.4 (s, C-arom), 130.4 (s, C-8), 119.2 (d, C-arom), 118.8 (d, C-arom) 111.8 (s, C-18), 83.6 (d, C-6), 82.1 (d, C-12), 81.1 (d, C-7), 75.2 (d, C-11), 59.7 (q, C-12 OMe), 57.3 (q, C-6 OMe), 37.7 (t, C-15), 35.8 (d, C-10), 31.8 (t, C-5), 31.2 (d, C-14), 30.8 (t, C-13), 24.5 (t, C-4), 19.3 (q, C-14 Me), 17.7 (q, C-10 Me), 13.8 (q, C-2 Me), 12.3 ppm (q, C-8 Me); HRMS [ESI] *m/z* for $C_{28}H_{42}N_2O_7Br$ $[M+H]^+$: calcd 597.2175, found 597.2175.

*18-Aza-reblastatin (**29**)*: ^{1}H NMR (500 MHz, *T* = 295 K, [D_4]methanol, [D_3]methanol = 3.31 ppm): *δ* 8.36 (s, 1H, 18-H), 8.12 (s, 1H, 17-H), 7.35 (s, 1H, 20-H), 5.80 (s, 1H, 3-H), 5.27 (d, 1H, *J* = 9.0 Hz, 9-H), 4.93-4.90 (m, 1H, 7-H), 3.57 (dd, 1H, *J* = 10.0, 3.3 Hz, 11-H), 3.43 (s, 3H, 12-OMe), 3.36 (s, 3H, 6-OMe) 3.31-3.28 (m, 1H, 6-H), 3.11 (dt, 1H, *J* = 10.2, 3.3 Hz, 12-H), 2.75 (dd, 1H, *J* = 13.6, 5.0 Hz, 15-H_a), 2.65 (dd, 1H, *J* = 13.6, 5.0 Hz, 15-H_b), 2.42 (dd, 1H, *J* = 10.0, 9.0 Hz, 10-H), 2.42-2.37 (m, 1H, 4-H_a), 2.32-2.27 (m, 1H, 4-H_b), 2.19-2.13 (m, 1H, 14-H), 1.87 (s, 3H, 2-Me), 1.67-1.59 (m, 2H, 13-H), 1.38 (s, 3H, 8-Me) 1.30-1.24 (m, 1H, 5-H_a), 1.20-1.13 (m, 1H, 5-H_b), 1.02 (d, 3H, *J* = 6.5 Hz, 10-Me) 0.81 ppm (d, 3H, *J* = 6.5 Hz, 14-Me); ^{13}C NMR (125 MHz, *T* = 295 K, [D_4]methanol, [D_4]methanol = 49.0 ppm): *δ* 171.1 (s, C-1), 159.1 (s, $COONH_2$), 147.5 (d, C-17), 141.3 (d, C-18), 138.0 (s, C-19), 137.6 (d, C-3), 135.3 (d, C-20), 134.5 (d, C-9), 132.2 (s, C-2), 131.4 (s, C-8), 130.5 (s, C-16), 83.5 (d, C-7), 81.7 (d, C-12), 81.0 (d, C-6), 75.0 (d, C-11), 59.8 (q, C-12 OMe), 57.3 (q, C-6 OMe), 40.4 (t, C-15), 35.9 (d, C-10), 31.1 (t, C-13), 31.0 (d, C-14), 30.3 (t, C-5), 24.4 (t, C-4), 18.5 (q, C-14 Me), 17.5 (q, C-10 Me), 13.7 (q, C-2 Me), 12.1 ppm (q, C-8 Me); HRMS [ESI] *m/z* for $C_{27}H_{42}N_3O_6$ $[M+H]^+$: calcd 504.3074, found 504.3065.

1.5 Cell proliferation assay

Cell lines were obtained from DMSZ (KB-3-1 ACC 158; U-937 ACC 5; A-431 ACC 91; A-549 ACC 107; MCF-7 ACC 115) or ATCC (SK-OV-3 HTB-77; PC-3 CRL-1435). Growth inhibition was measured in microtiter plates. Sixty µL of serial dilutions of the test compounds were added to 120 µL aliquots of a cell suspension (50.000/mL) in 96-well plates and incubated at 37 ℃ under 10% CO_2 for 5 days. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to measure growth

and viability of cells which are capable of reducing it to a violet formazan product. 20 µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg/mL. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL isopropanol containing 0.4% hydrochloric acid. The microplates were measured at 595 nm using an ELISA plate reader. All experiments were carried out in two parallel experiments, the percentage of viable cells was calculated as the mean with respect to the controls set to 100%. In the case of U-937 the WST-1 assay from Roche was employed.

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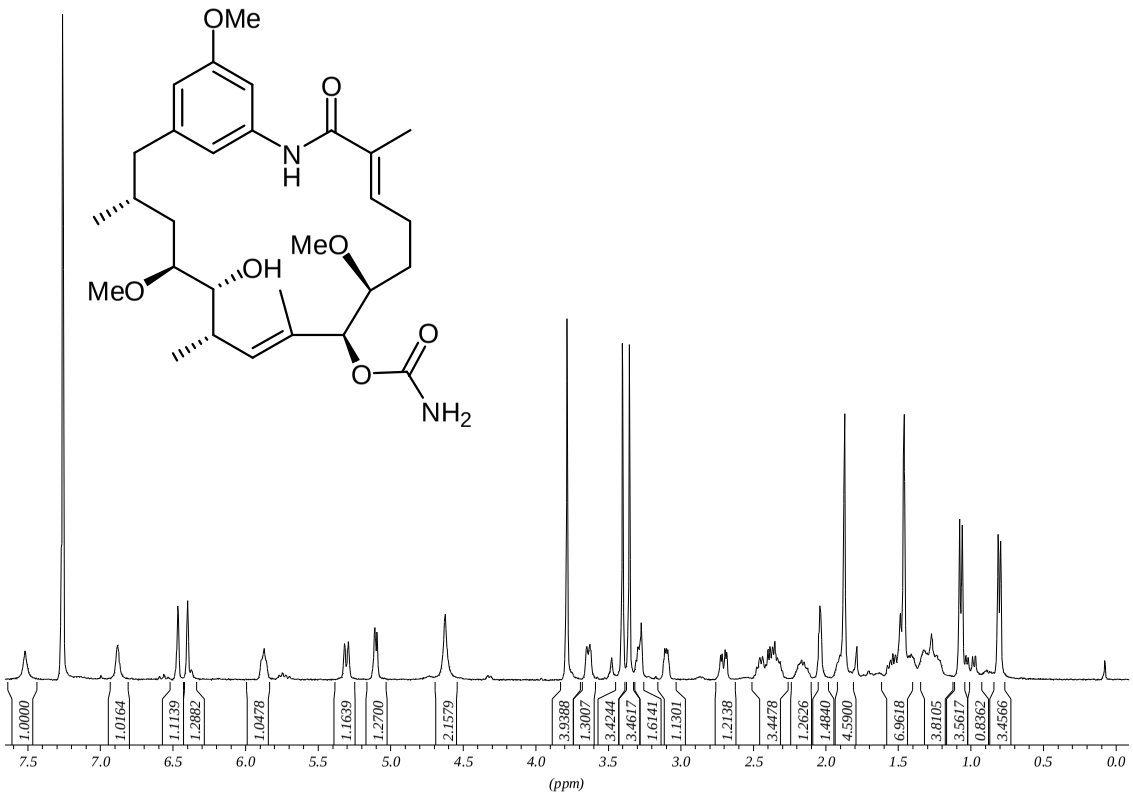
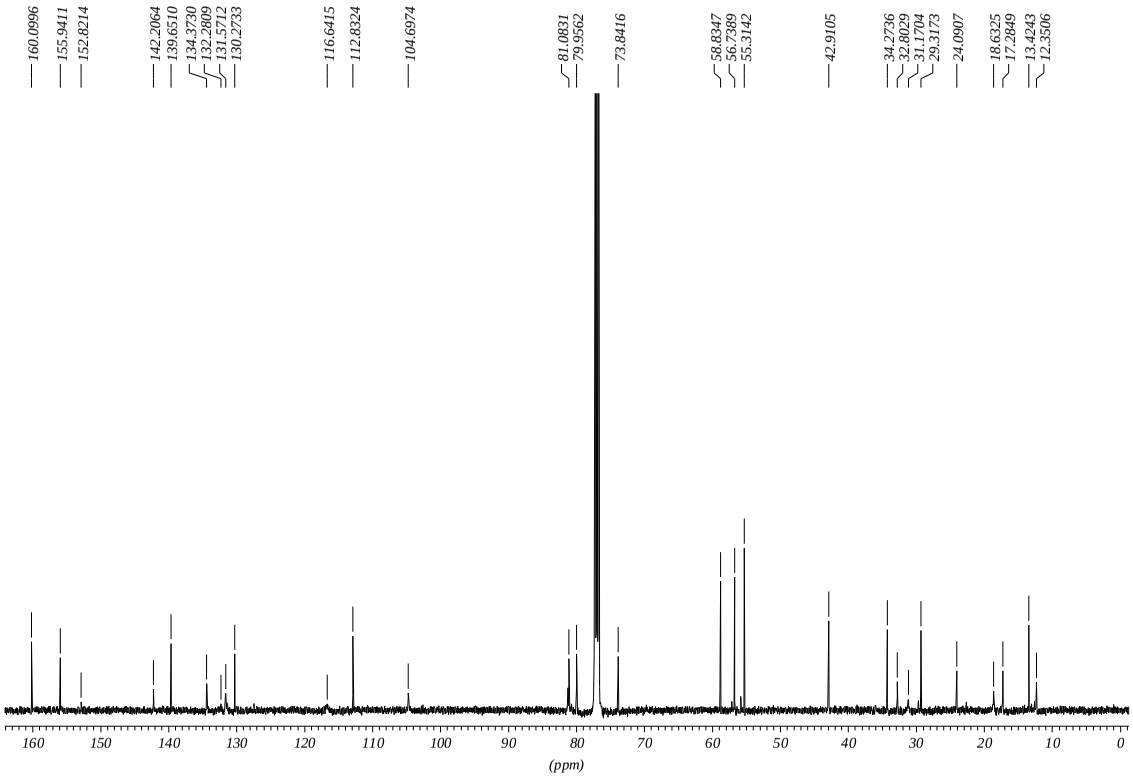
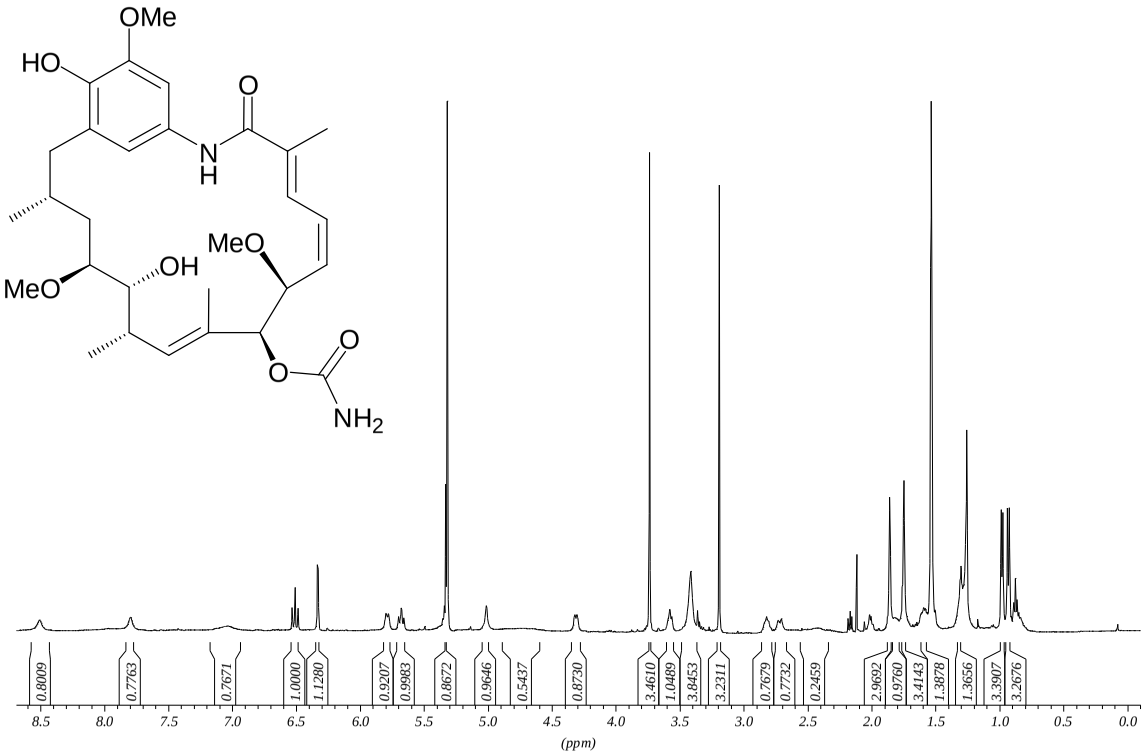
^{1}H NMR and ^{13}C NMR spectra






OMe
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H
MeO
OH
MeO
O
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0.9983
0.8672
0.9646
0.5437
0.8730
3.4610
1.0489
3.8453
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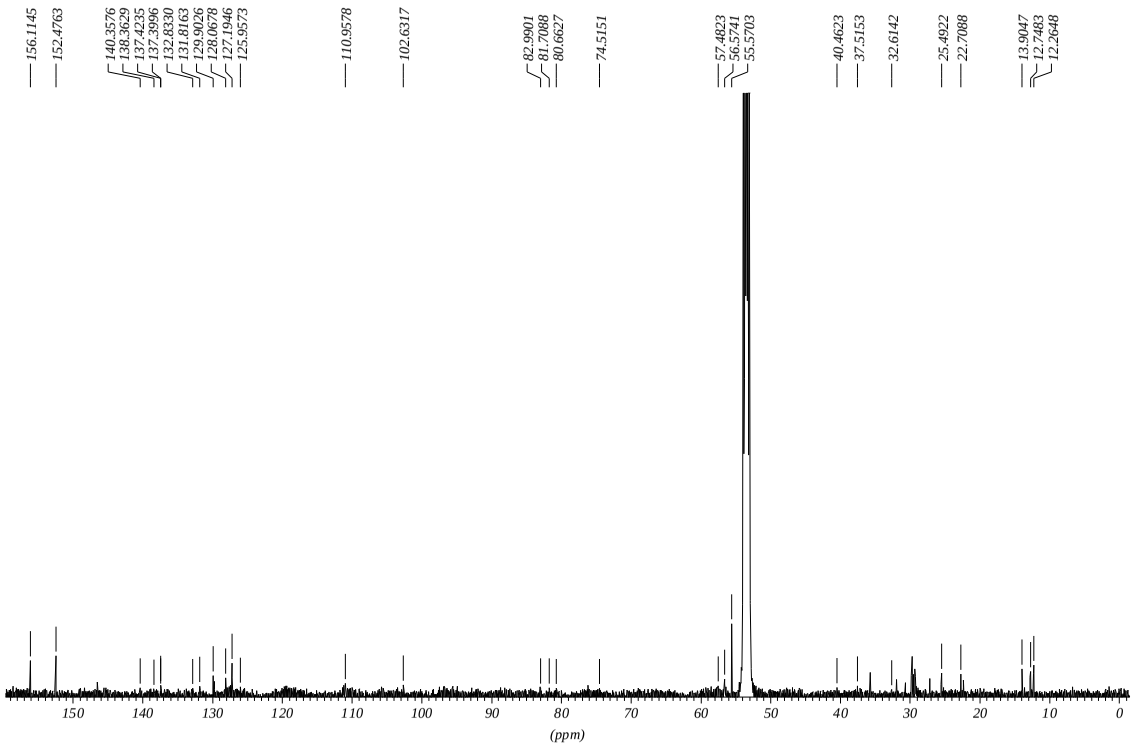
0.7679
0.7732
0.2459
2.9692
0.9760
3.4143
1.3878
1.3656
3.3907
3.2676
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
(ppm)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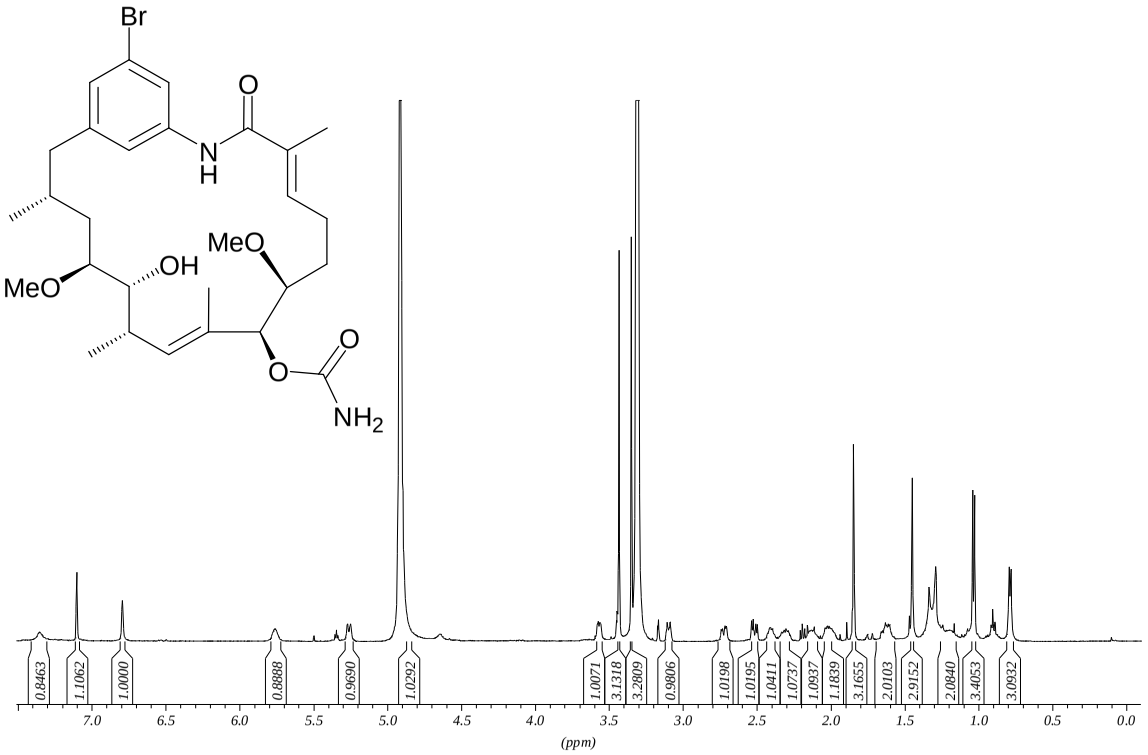
138.3629
137.4235
137.3996
132.8330
131.8163
129.9026
128.0678
127.1946
125.9573
110.9578
102.6317
82.9901
81.7088
80.6627
74.5151
57.4823
56.5741
55.5703
40.4623
37.5153
32.6142
25.4922
22.7088
13.9047
12.7483
12.2648
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)

MeO
OH
MeO
N
H
F
O
O
O
NH2

0.8800
0.9861
1.0000
1.0063
0.9500
1.0197
0.8638
1.9735
1.0399
2.9686
2.9075
1.0106
1.0192
1.0216
1.0669
1.0042
1.1524
1.0708
1.1638
2.9549
1.1444
2.9190
2.0358
1.0858
3.1537
2.7697
(ppm)

171.8437
160.0654
157.6477
156.2721
138.8561
135.0170
134.6683
131.7514
130.2805
128.8744
128.1161
127.9371
121.4058
121.3220
115.8882
115.6443
81.3135
81.0886
80.3666
74.1249
58.8581
56.9090
35.0633
34.5203
33.0494
31.3804
29.5990
24.4986
18.6095
17.4511
13.6177
12.6136
(ppm)


Br
O
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H
MeO
OH
MeO
O
O
NH2
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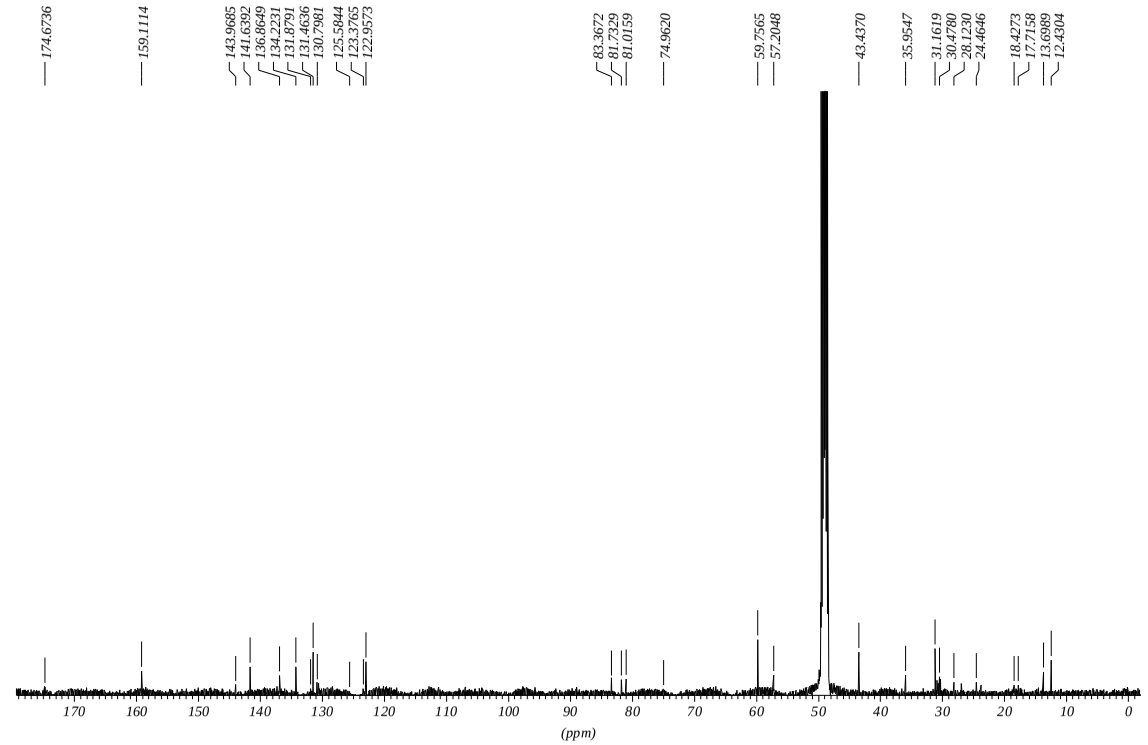
1.0000
0.8888
0.9690
1.0292
1.0071
3.1318
3.2809
0.9806
1.0198
1.0195
1.0411
1.0737
1.0937
1.1839
3.1655
2.0103
2.9152
2.0840
3.4053
3.0932
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)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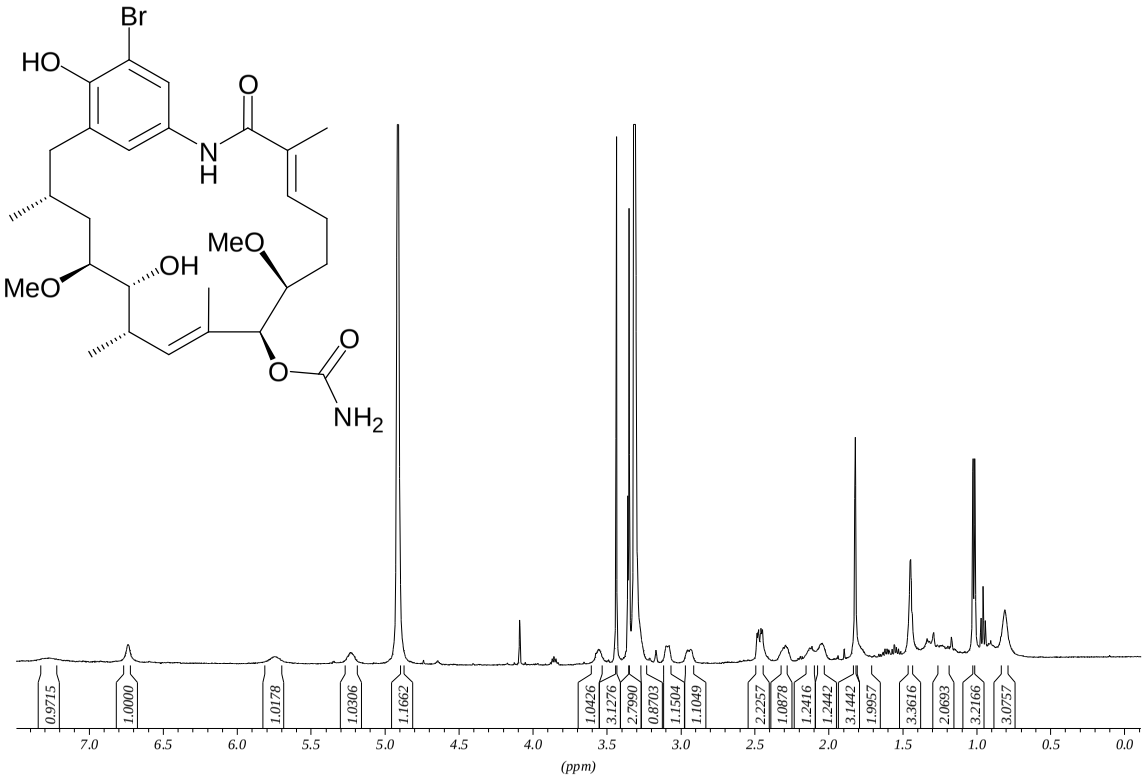
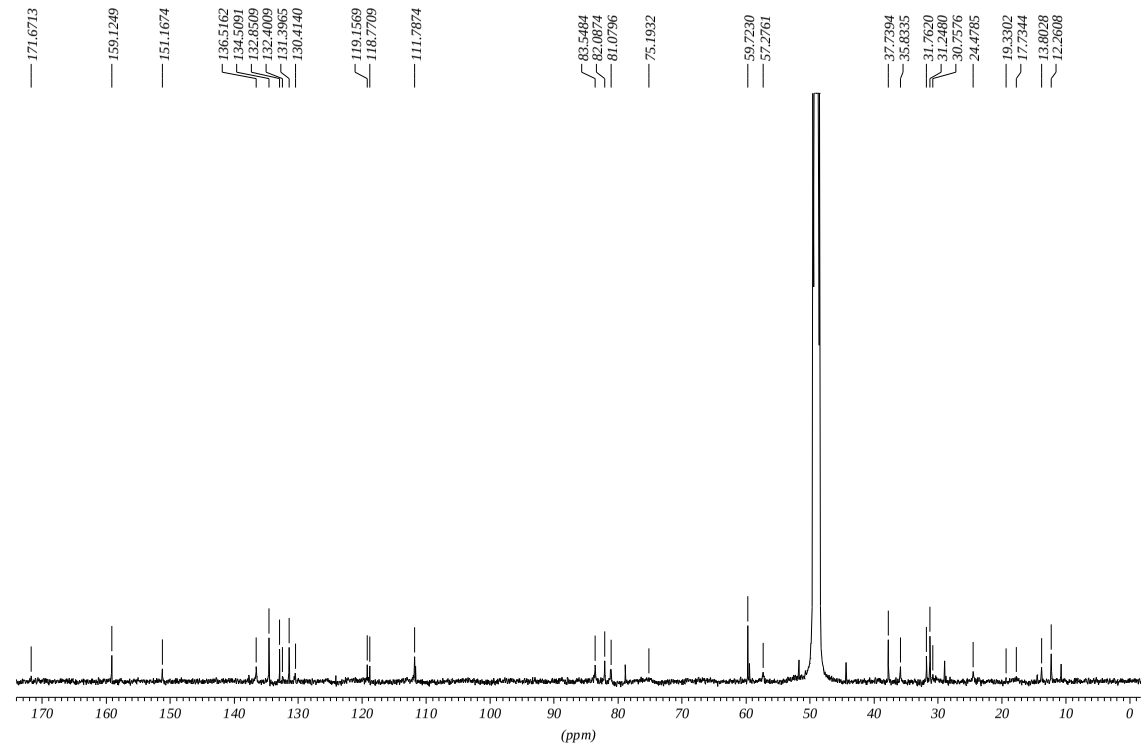
141.6392
136.8649
134.2231
131.8791
131.4636
130.7981
125.5844
123.3765
122.9573
83.3672
81.7329
81.0159
74.9620
59.7565
57.2048
43.4370
35.9547
31.1619
30.4780
28.1230
24.4646
18.4273
17.7158
13.6989
12.4304
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


Br
HO
O
N
H
MeO
OH
MeO
O
O
NH2
0.9715
1.0000
1.0178
1.0306
1.1662
1.0426
3.1276
2.7990
0.8703
1.1504
1.1049
2.2257
1.0878
1.2416
1.2442
3.1442
1.9957
3.3616
2.0693
3.2166
3.0757
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)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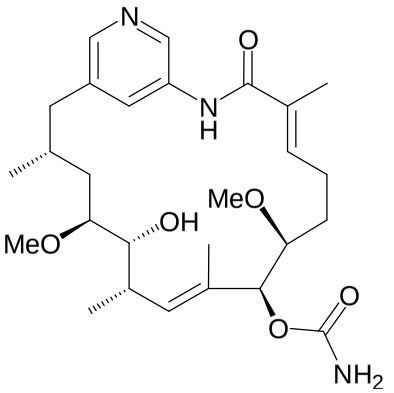
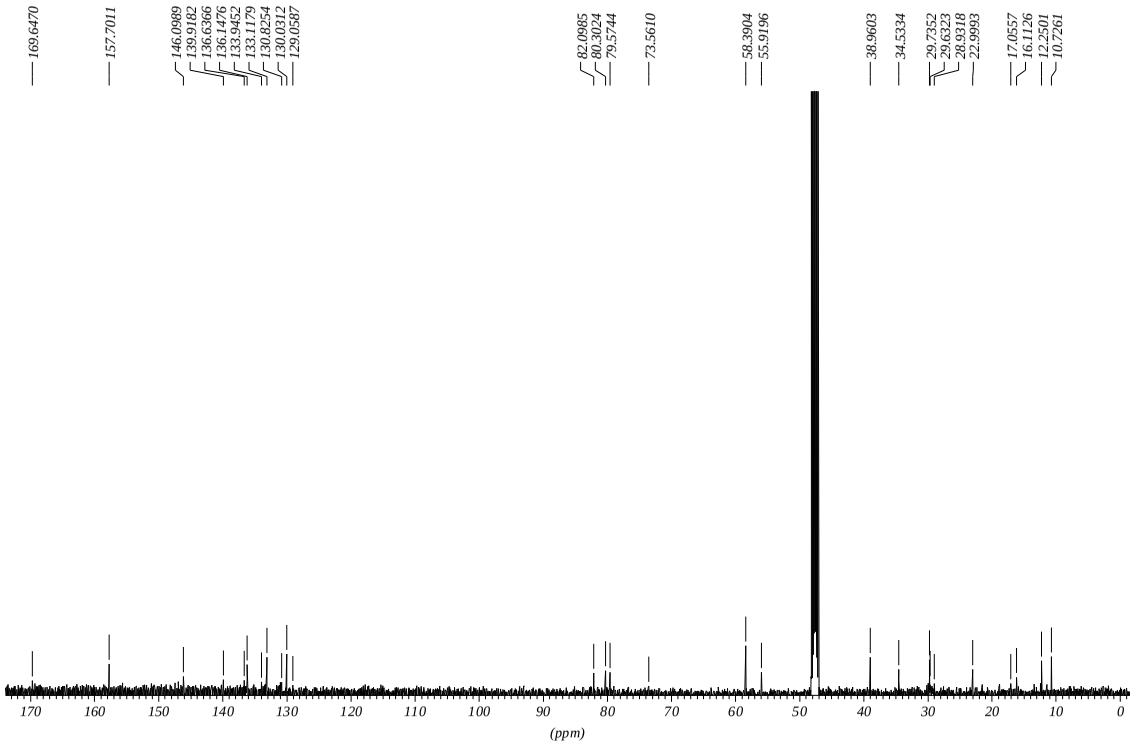
159.1249
151.1674
136.5162
134.5091
132.8509
132.4009
131.3965
130.4140
119.1569
118.7709
111.7874
83.5484
82.0874
81.0796
75.1932
59.7230
57.2761
37.7394
35.8335
31.7620
31.2480
30.7576
24.4785
19.3302
17.7344
13.8028
12.2608
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


N
O
N
H
MeO
OH
MeO
O
O
NH2

1.0199
0.9705
1.0000
1.0315
1.0704
1.1361
1.1331
3.3092
3.3388
0.6958
1.1164
1.0085
1.0457
0.9883
1.0456
1.1352
1.2084
3.0442
1.1554
0.9763
3.0590
0.9294
1.0570
2.9608
3.0874
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)


169.6470
157.7011
146.0989
139.9182
136.6366
136.1476
133.9452
133.1179
130.8254
130.0312
129.0587
82.0985
80.3024
79.5744
73.5610
58.3904
55.9196
38.9603
34.5334
29.7352
29.6323
28.9318
22.9993
17.0557
16.1126
12.2501
10.7261
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)