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SUPPLEMENTARY INFORMATION

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Synthesis of marmycin A and investigation into its cellular activity

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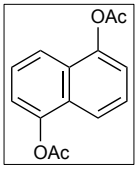
Contents

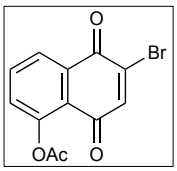
1. Synthesis

All starting materials were purchased from commercial sources and used without further purification, or purified according to *Purification of Laboratory Chemicals* (Armarego, W.L.F., Chai, C.L.L. 5th edition). Solvents were dried under standard conditions. Reactions were monitored by thin-layer chromatography (TLC) using TLC silica gel coated aluminum plates 60F-254 (Merck). Column chromatography was performed using Merck silica gel 60, 0.040-0.063 mm (230-400 mesh). Preparative TLC were carried out using 0.50 mm Merck silica gel plates (60F-254). NMR spectroscopy was performed on Bruker 300, 500, 600 and 950 MHz apparatus equipped with a cryoprobe. Spectra were run in $CDCl_3$ at 298 K unless otherwise stated. Molecular structures have been characterized using a comprehensive dataset including ^{1}H- and ^{13}C-NMR spectra (1D and 2D experiments). ^{1}H chemical shifts are expressed in ppm using the residual non deuterated solvent as internal standard ($CDCl_3$ ^{1}H, 7.26 ppm). The following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet. ^{13}C chemical shifts are expressed in ppm using the residual non deuterated solvent as internal standard ($CDCl_3$ ^{13}C, 77.16 ppm). Exact masses were recorded on a LCT Premier XE (Waters) equipped with an ESI ionization source and a TOF detector and on a Q-ToF 6540 (Agilent). Optical rotation measurements were performed on an Anton Paar Polarimeter MCP 300. The following parameters used were: temperature 20 °C, 100 mm path-length quartz cuvette, wavelength 589 nm. UV/vis absorption spectra were recorded on a Cary 5000 spectrophotometer from Agilent Technologies. Corrected emission spectra were obtained on a Fluorolog FL3-221 spectrofluorometer from Horiba Jobin-Yvon. Measurements were collected using a 1 cm path-length quartz cuvette. The following parameters used were: bandwidth 1 nm, response time 1 sec, wavelength scan range 200–800 nm.

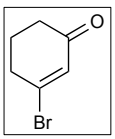
Experimental procedures



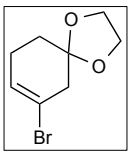
Naphthalene-1,5-diol diacetate (22). Compound **22** was prepared according to a previously published procedure[1]. A mixture of naphthalene-1,5-diol (5.0 g, 31.2 mmol), acetic anhydride (6.5 mL, 68.7 mmol), and pyridine (5.5 mL, 68.7 mmol) in dichloromethane (300 mL) was stirred at room temperature for 2 h. After this time, the reaction mixture was quenched with sat. aq. $NaHCO_3$ and extracted with dichloromethane. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (heptane/ethyl acetate 1:0 to 1:1) to afford **22** (5.8 g, 76%) as a dark solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.5 Hz, 2H), 7.51 (dd, *J* = 8.5, 7.5 Hz, 2H), 7.29 (d, *J* = 7.5 Hz, 2H), 2.47 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 146.9, 128.3, 126.2, 119.4, 118.9, 21.2. HRMS (APPI) calcd. for $C_{14}H_{13}O_4^+$ $[M+H]^+$ 245.0808, found: 245.0812.



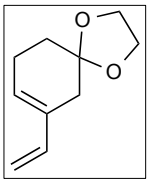
5-Acetoxy-2-bromonaphthalene-1,4-dione (9). Compound **9** was prepared according to a previously published procedure[2]. A solution of **22** (5.8 g, 23.8 mmol) in acetic acid (180 mL) was added dropwise to a solution of *N*-bromosuccinimide (16.9 g, 95.1 mmol) in acetic acid/water (540 mL, 1:2) and the reaction mixture was stirred at 65 ºC for 1 h. After this time, the reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The resulting dark solid was recrystallized from ethanol to afford **9** as orange needles (4.2 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.77 (t, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.39 (s, 1H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.1, 177.6, 169.4, 150.0, 141.6, 138.7, 135.1, 132.7, 130.5, 126.5, 123.3, 21.2. HRMS (APPI) calcd. for $C_{12}H_8BrO_4^+$ $[M+H]^+$ 294.9600, found: 294.9603.



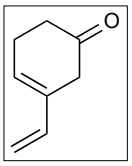
3-Bromocyclohex-2-en-1-one (23). Compound **23** was prepared according to a previously published procedure[3]. A flask loaded with triphenylphosphine (37.5 g, 143.1 mmol) and benzene (300 mL) was cooled to 0 ºC followed by the dropwise addition of bromine (7.4 mL, 143.1 mmol) in benzene (50 mL) and the reaction mixture was allowed to warm to room temperature over 1 h. After this time, triethylamine (20 mL, 143.1 mmol) was added followed by a solution of 1,3-cyclohexanedione (10 g, 89.3 mmol) in chloroform (100 mL) and the mixture was stirred at room temperature for another 3 h. The mixture was then filtered through celite, washed with water and brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 7:3) to afford **23** (11.54 g, 74%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (t, *J* = 1.5 Hz, 1H), 2.82 (td, *J* = 6.0, 1.5 Hz, 2H), 2.43–2.38 (m 2H), 2.12–2.03 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.4, 150.2, 132.7, 36.5, 36.4, 23.1. HRMS (APPI) calcd. for $C_6H_8BrO^+$ $[M+H]^+$ 174.9753, found: 174.9744.



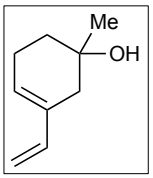
1-Bromo-5-dioxolanecyclohex-1-ene (24). Compound **24** was prepared according to a previously published procedure[3]. A flask equipped with a Dean Stark apparatus was loaded with **23** (5.75 g, 33.0 mmol), ethylene glycol (3.3 mL, 59.1 mmol), *p*-toluenesulfonic acid (125 mg, 0.66 mmol) and benzene (820 mL). The reaction mixture was refluxed for 2 h with azeotropic removal of water. The mixture was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 7:3) to afford **24** (3.96 g, 55%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.28–5.95 (m, 1H), 4.13–3.90 (m, 4H), 2.65 (d, *J* = 2.0 Hz, 2H), 2.37–2.16 (m, 2H), 1.74 (t, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.1, 117.7, 108.1, 64.7 (2C), 45.5, 30.3, 25.5. HRMS (APPI) calcd. for $C_8H_{12}BrO_2^+$ $[M+H]^+$ 219.0015, found: 219.0015.



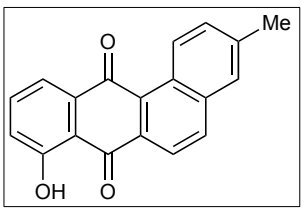
5-Dioxolane-1-vinylcyclohex-1-ene (25). Compound **25** was prepared according to a previously published procedure[4]. In a Schlenk tube, a solution of vinylmagnesium bromide (1.0 M in tetrahydrofurane, 60 mL, 60.3 mmol) was added to a mixture of **24** (4 g, 18.3 mmol) and $Pd(PPh_3)_4$ (1 g, 0.9 mmol) in tetrahydrofurane (60 mL) at room temperature. The reaction mixture was refluxed for 30 min, then quenched with sat. aq. NH_4Cl and extracted with *tert*-butylmethylether. The resulting organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 7:3) to afford **25** (2.5 g, 82%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.40 (dd, *J* = 17.5, 10.5 Hz, 1H), 5.77 (bs, 1H), 5.03 (d, *J* = 17.5 Hz, 1H), 4.92 (d, *J* = 10.5 Hz, 1H), 4.15–3.91 (m, 4H), 2.39–2.35 (m, 4H), 1.77 (t, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.3, 134.3, 128.3, 110.6, 108.5, 64.6 (2C), 34.7, 31.1, 24.7. HRMS (APPI) calcd. for $C_{10}H_{15}O_2^+$ $[M+H]^+$ 167.1067, found: 167.1067.



3-Vinyl-3-cyclohex-1-one (26). Compound **26** was prepared according to a previously published procedure[4]. To a solution of **25** (2.47 g, 14.88 mmol) in water (23 mL) was added $LiBF_4$ (1 M in acetonitrile, 64 mL, 63.98 mmol). The mixture was stirred at room temperature overnight and extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 7:3) to afford **26** (1.3 g, 71%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.43 (dd, *J* = 17.0, 11.0 Hz, 1H), 5.95 (bs, 1H), 5.04 (d, *J* = 17.0, 1H), 5.02 (d, *J* = 11.0 Hz, 1H), 3.00 (d, *J* = 1.0 Hz, 2H), 2.58–2.57 (m, 2H), 2.51 (t, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.6, 137.4, 134.0, 127.5, 111.3, 38.4, 37.8, 24.8. HRMS (APPI) calcd. for $C_8H_{11}O^+$ $[M+H]^+$ 123.0804, found: 123.0805.

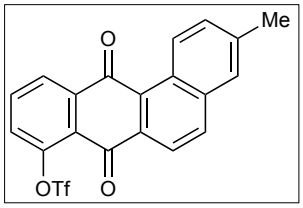


1-Methyl-3-vinyl-3-cyclohexen-1-ol (10). Compound **10** was prepared according to a modified procedure[4]. To a Schlenk tube containing dry $CeCl_3$ (13.7 g, 55.6 mmol) was added freshly distilled tetrahydrofurane (80 mL) and the solution was stirred at room temperature for 2 h. A solution of MeMgBr (1.4 M in diethyl ether, 40 mL, 55.6 mmol) was added dropwise at 0 °C and the reaction was stirred for another 2 h. A solution of **26** (2.5 g, 20.5 mmol) in tetrahydrofurane (10 mL) was then added dropwise and the reaction was stirred at 0 °C for 30 min. After this time, the reaction mixture was quenched with sat. aq. NH_4Cl and extracted with *tert*-butylmethylether. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 7:3) to afford **10** (2.2 g, 79%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.39 (dd, *J* = 17.5, 10.5 Hz, 1H), 5.76 (bs, 1H), 5.07 (d, *J* = 17.5 Hz, 1H), 4.92 (d, *J* = 10.5 Hz, 1H,), 2.40–1.53 (m, 7H), 1.31 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.6, 134.2, 128.2, 110.5, 68.9, 38.5, 35.1, 28.9, 23.5.

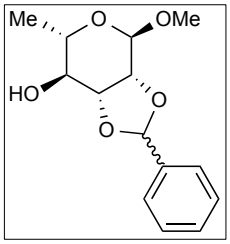


8-hydroxy-3-methyltetraphene-7,12-dione (14). A solution of **9** (3.26 g, 11.1 mmol) and **10** (1.52 g, 11.1 mmol) in toluene (35 mL) was stirred at 100 °C for 16 h. The solvent was removed under reduced pressure, then methanol (30 mL) was added and the reaction mixture was stirred at room temperature for 4 h in the dark. Potassium carbonate (4.6 g, 33.3 mmol) was added and the reaction was stirred at room temperature for 1 h. The mixture was neutralized with aq. HCl (1 N) and extracted with dichloromethane. The combined organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether/*tert*-butylmethylether, 1:0 to 9:1) to afford **14** (1 g, 33%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.39 (s, 1H), 9.52 (d, *J* = 9.0 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 8.05 (d, *J* =

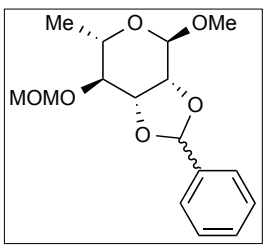
8.5 Hz, 1H), 7.77 (d, *J* = 7.5 Hz, 1H), 7.65–7.62 (m, 2H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.9, 185.4, 161.6, 139.4, 137.2, 136.6, 134.9, 134.6, 133.9, 132.4, 129.5, 128.6, 128.4, 127.8, 123.4, 121.9, 119.5, 115.2, 21.6. HRMS (APPI) calcd. for $C_{19}H_{13}O_3^+$ $[M+H]^+$ 289.0859, found: 289.0868.



3-methyl-7,12-dioxo-7,12-dihydrotetraphen-8-yl trifluoromethanesulfonate (7). A solution of **14** (62 mg, 0.217 mmol) in dichloromethane (9 mL) was cooled to –78 °C prior the addition of triethylamine (0.12 mL, 0.868 mmol) and the reaction mixture was stirred for 30 min. Triflic anhydride (0.14 mL, 0.868 mmol) was then added dropwise and the reaction was allowed to warm to room temperature over 6 h. The mixture was diluted with dichloromethane and washed with sat. aq. $NaHCO_3$. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (heptane/ethyl acetate, 8:2) to afford **7** (71 mg, 78%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.46 (d, *J* = 8.5 Hz, 1H), 8.39 (d, *J* = 8.0 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.67 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 2.55 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.2, 181.8, 146.9, 139.6, 137.2, 137.0, 135.3, 135.0, 133.7, 132.7, 128.3, 128.2, 128.1, 127.9, 127.6, 124.7, 122.7, 120.0, 117.5, 21.6. HRMS (APPI) calcd. for $C_{20}H_{12}F_3O_5S^+$ $[M+H]^+$ 421.0352, found: 421.0354.

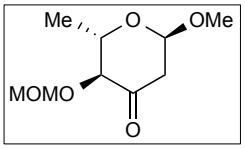


Methyl 2,3-*O*-benzylidene-6-deoxy-α-L-mannopyranoside (27). Compound **27** was prepared according to a previously published procedure[5]. A mixture of methyl α-L-rhamnopyranoside (10 g, 56.1 mmol), benzaldehyde dimethyl acetal (10.11 mL, 67.3 mmol), and *p*-toluenesulfonic acid (0.27 g, 1.4 mmol) was refluxed in dry dimethylformamide (11 mL) under reduced pressure (water aspirator vacuum) overnight. The reaction mixture was neutralized with sat. aq. $NaHCO_3$ and extracted with *tert*-butylmethylether. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The yellow mixture of isomers (14.62 g, 98%) was used in the next step without further purification. Mixture of isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53–7.45 (m, 2H), 7.45–7.31 (m, 8H), 6.07 (s, 1H), 5.86 (s, 1H), 4.98 (s, 1H), 4.90 (s, 1H), 4.37 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.25–4.14 (m, 2H), 4.07 (d, *J* = 5.5 Hz, 1H), 3.72–3.59 (m, 2H), 3.55 (d, *J* = 4.5 Hz, 1H), 3.49 (d, *J* = 4.5 Hz, 1H), 3.47–3.37 (m, 2H), 3.36 (s, 3H), 3.33 (s, 3H), 1.31 (d, *J* = 6.5 Hz, 3H), 1.23 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 136.9, 129.4, 129.1, 128.4, 128.3, 126.7, 126.1, 104.0, 102.7, 97.9, 97.8, 79.7, 78.2, 77.9, 75.3, 74.4, 71.5, 65.6, 65.2, 54.8, 54.7, 17.3, 17.3. HRMS (ESI-TOF) calcd. for $C_{14}H_{19}O_5^+$ $[M+H]^+$ 267.1227, found: 267.1219.

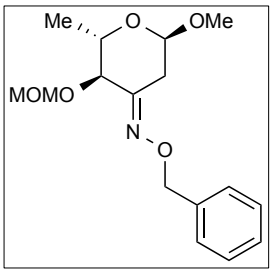


Methyl 2,3-*O*-benzylidene-6-deoxy-4-*O*-(methoxymethyl)-α-L-mannopyranoside (15). Compound **15** was prepared according to a previously published procedure[6]. To a solution of **27** (3.6 g, 13.5 mmol) and Hünig´s base (4.71 mL, 27.1 mmol) in dichloromethane (50 mL) was slowly added chloromethyl methyl ether (2.05 mL, 27.1 mmol) at room temperature. The reaction mixture was stirred for 72 h prior addition of sat. aq. $NaHCO_3$ and was extracted with *tert*-butylmethylether. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography (heptane/ethyl acetate, 8:2) to afford **15** (3.8 g, 91%) as a colorless oil. Mixture of isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 2H), 7.48–7.33 (m, 8H), 6.14 (s, 1H), 5.91 (s, 1H), 5.03 (d, *J* = 6.5 Hz, 1H), 4.98 (s, 1H), 4.92 (s, 1H), 4.88 (d, *J* = 6.5 Hz, 1H), 4.71 (d, *J* = 6.5 Hz, 1H), 4.67 (d, *J* = 6.5 Hz, 1H), 4.49 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.32 (t, *J* = 6.5 Hz, 1H), 4.19 (d, *J* = 6.5 Hz, 1H), 4.11 (d, *J* = 5.5 Hz, 1H), 3.77–3.67

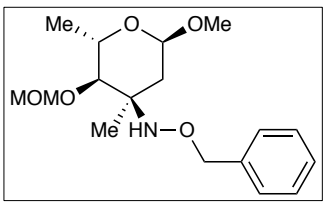
(m, 2H), 3.57 (dd, *J* = 10.0, 7.5 Hz, 1H), 3.50–3.45 (m, 1H), 3.44 (s, 3H), 3.40 (s, 3H), 3.38 (s, 3H), 3.37 (s, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.31 (d, *J* = 6.5 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 138.7, 137.2, 129.4, 129.3, 128.5, 128.5, 126.8, 126.3, 104.2, 102.8, 98.1, 97.9, 96.4, 96.4, 79.5, 79.0, 78.3, 77.9, 75.6, 75.3, 64.4, 64.2, 56.0, 55.9, 55.0, 54.9, 17.9, 17.8. HRMS (ESI-TOF) calcd. for $C_{16}H_{23}O_6^+$ $[M+H]^+$ 311.1489, found: 311.1490.



Methyl 2,6-dideoxy-4-*O*-(methoxymethyl)-α-L-erythrohexopyranosid-3-dose (28). Compound **28** was prepared according to a modified procedure[6]. A solution of **15** (5 g, 16.1 mmol) in freshly distilled tetrahydrofurane (200 mL) was cooled to –40 ºC under argon prior dropwise addition of *sec*-buthyllithium (1.6 M in hexane, 30 mL, 48.3 mmol). After 1.5 h, the mixture was poured into ice-water containing NH_4Cl and the organic material was extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography (heptane/ethyl acetate, 7:3) to afford **28** (2 g, 61%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (d, *J* = 4.5 Hz, 1H), 4.81 (d, *J* = 7.0 Hz, 1H), 4.67 (d, *J* = 7.0 Hz, 1H), 4.04–3.83 (m, 2H), 3.41 (s, 3H), 3.31 (s, 3H), 2.73 (ddd, *J* = 14.0, 4.5, 1.0 Hz, 1H), 2.56 (dd, *J* = 14.0, 1.0 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 203.0, 99.6, 96.5, 81.6, 69.2, 56.3, 54.9, 46.9, 18.9. HRMS (ESI-TOF) calcd. for $C_9H_{16}O_5Na^+$ $[M+Na]^+$ 227.0890, found: 227.0893.

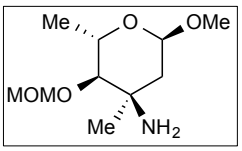


Methyl 4-*O*-methoxymethyl-2,6-dideoxy-α-L-threo-hexopyranosid-3-ulose *O*-benzyloxime (16). To a solution of **28** (0.30 g, 1.47 mmol) in ethanol (30 mL) was added *O*-benzylhydroxylamine hydrochloride (0.70 g, 4.41 mmol) and sodium acetate (0.80 g, 5.88 mmol). After stirring at room temperature for 2 h, the mixture was filtered, concentrated to dryness under reduced pressure and purified by flash chromatography (heptane/ethyl acetate, 7:3) to afford **16** (0.41 g, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.27 (m, 5H), 5.14 (s, 2H), 4.77–4.57 (m, 2H), 4.60 (d, *J* = 7.0 Hz, 1H), 4.04–3.91 (m, 1H), 3.85 (d, *J* = 8.5 Hz, 1H), 3.36 (s, 3H), 3.34 (s, 3H), 3.23 (dd, *J* = 14.5, 2.5 Hz, 1H), 2.38 (dd, *J* = 14.5, 4.5 Hz, 1H), 1.34 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.7, 138.2, 128.4, 128.2, 127.8, 97.7, 95.7, 76.0, 75.9, 68.9, 56.2, 54.9, 30.6, 18.3. HRMS (ESI-TOF) calcd. for $C_{16}H_{24}NO_5^+$ $[M+H]^+$ 310.1649, found: 310.1653. $[\alpha]_D^{20}$ –239 (*c* 0.072, $CHCl_3$).

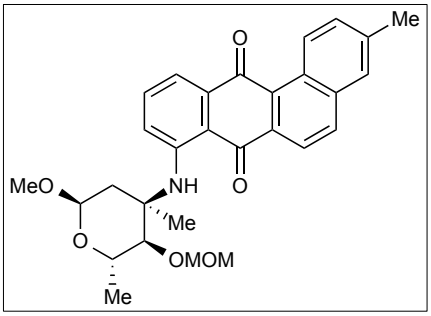


***O*-benzyl-*N*-((2*S*,3*R*,4*R*,6*R*)-6-methoxy-3-(methoxymethoxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)hydroxylamine (29).** To a Schlenk tube containing dry $CeCl_3$ (3.98 g, 16.2 mmol) was added freshly distilled tetrahydofurane (50 mL) under argon and the solution was stirred at room temperature overnight. The reaction was cooled to –78 ºC prior dropwise addition of methyllithium (3 M in diethoxymethane, 4.32 mL, 13.0 mmol) and the reaction mixture was stirred for 1 h. After this time, a solution of **16** (1 g, 3.2 mmol) in freshly distilled tetrahydrofurane (10 mL) was added and the reaction was stirred at –78 ºC for another 2 h. The reaction mixture was allowed to warm to room temperature, quenched with sat. aq. $NaHCO_3$ and extracted with *tert*-butylmethylether. The combined organic layer was dried over $MgSO_4$, concentrated to dryness under reduced pressure and purified by flash chromatography (heptane/ethyl acetate, 8:2) to afford **29** (0.76 g, 72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.26 (m, 5H), 6.27 (bs, 1H), 4.87–4.86 (m, 5H), 3.97–3.90 (m, 1H), 3.39 (s, 3H), 3.35 (s, 3H), 3.15 (d, *J* = 9.0 Hz, 1H), 2.19 (dd, *J* = 14.5, 2.0 Hz, 1H), 1.56 (dd, *J* = 14.5, 5.0 Hz, 1H), 1.28 (s, 3H), 1.25 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 128.5, 128.3, 127.6, 98.9, 98.4, 85.6, 76.9, 64.5, 58.6, 56.5, 55.1, 36.5, 24.0,

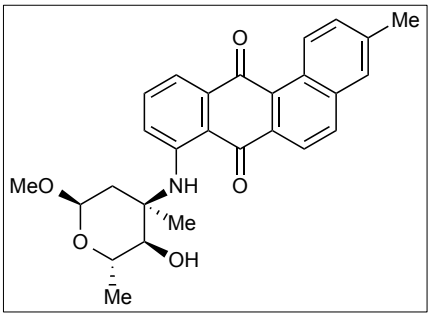
18.7. HRMS (ESI-TOF) calcd. for $C_{17}H_{28}NO_5^+$ $[M+H]^+$ 326.1962, found: 326.1970. $[\alpha]_D^{20}$ –90 (*c* 0.123, $CHCl_3$).



(2*S*,3*R*,4*R*,6*R*)-6-methoxy-3-(methoxymethoxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-amine (8). A solution of **29** (0.230 g, 0.71 mmol) and Pd/C 10% (0.034 g, 15% wt) in methanol (7 mL) was stirred at room temperature overnight under hydrogen atmosphere. The reaction mixture was filtered through celite and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography (dichloromethane/methanol, 9:1) to afford **8** (0.136 g, 88%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75 (d, *J* = 6.5 Hz, 1H), 4.68 (d, *J* = 6.5 Hz, 1H), 4.64 (d, *J* = 4.0 Hz, 1H), 3.93–3.66 (m, 3H), 3.42 (s, 3H), 3.31 (s, 3H), 3.02 (d, *J* = 9.5 Hz, 1H), 2.03 (d, *J* = 14.5 Hz, 1H), 1.68 (dd, *J* = 14.5, 4.0 Hz, 1H), 1.27 (d, *J* = 6.5 Hz, 3H), 1.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.9, 98.2, 85.0, 63.5, 56.7, 55.0, 52.2, 40.8, 28.3, 18.2. HRMS (ESI-TOF) calcd. for $C_{10}H_{22}NO_4^+$ $[M+H]^+$ 220.1543, found: 220.1542. $[\alpha]_D^{20}$ –130 (*c* 0.137, $CHCl_3$).

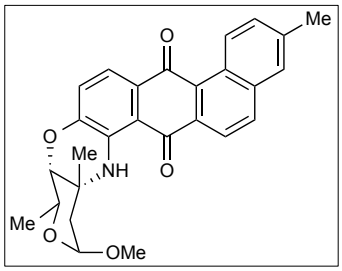


8-(((2*S*,3*R*,4*R*,6*R*)-6-methoxy-3-(methoxymethoxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)amino)-3-methyltetraphene-7,12-dione (17). A dry sealed tube was loaded with **7** (34.0 mg, 0.081 mmol), CuI (3.1 mg, 20 mol %), potassium carbonate (22.3 mg, 0.162 mmol), amine **8** (88.6 mg, 0.405 mmol) and the mixture was stirred in dry toluene (4 mL) at 160 ºC for 72 h. The reaction was then cooled to room temperature and washed with sat. aq. $NaHCO_3$. The combined organic layer was dried over $MgSO_4$, concentrated to dryness under reduced pressure and purified by flash chromatography (heptane/ethyl acetate, 8:2) to afford **17** (13.05 mg, 33%) as a dark violet solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.41 (bs, 1H), 9.56 (d, *J* = 9.0 Hz, 1H), 8.44 (d, *J* = 8.5 Hz, 1H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.66 (s, 1H), 7.59–7.50 (m, 2H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 9.0 Hz, 1H), 4.91–4.87 (m, 2H), 4.64 (d, *J* = 3.5 Hz, 1H), 4.32 (dq, *J* = 9.0, 6.0 Hz, 1H), 3.49 (s, 3H), 3.28 (d, *J* = 9.0 Hz, 1H), 3.14 (s, 3H), 2.77 (d, *J* = 15.0 Hz, 1H), 2.55 (s, 3H), 1.83 (dd, *J* = 15.0, 3.5 Hz, 1H), 1.66 (s, 3H), 1.40 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.3, 185.1, 151.0, 138.4, 137.1, 136.4, 135.5, 134.2, 134.2, 131.9, 128.8, 128.5, 128.4, 127.7, 123.0, 120.5, 115.6, 113.8, 99.0, 97.5, 87.4, 64.4, 56.8, 55.7, 54.9, 38.3, 26.5, 21.7, 18.7. HRMS (ESI-TOF) calcd. for $C_{29}H_{32}NO_6^+$ $[M+H]^+$ 490.2224, found: 490.2225. $[\alpha]_D^{20}$ –464 (*c* 0.003, $CHCl_3$).

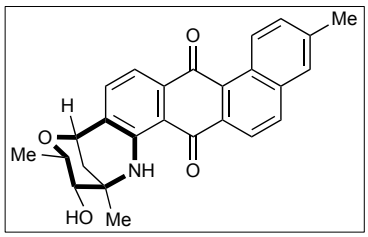


8-(((2*S*,3*R*,4*R*,6*R*)-3-hydroxy-6-methoxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)amino)-3-methyltetraphene-7,12-dione (18). To a solution of **17** (10 mg, 0.020 mmol) in dry dichloromethane (1 mL) was added $BF_3{\cdot}OEt_2$ (4.3 µL, 0.040 mmol) at –78 ºC. The reaction mixture was allowed to warm to room temperature and was stirred for 24 h. After this time, the reaction was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude reaction product was purified by preparative TLC (petroleum ether/*tert*-butylmethylether, 1:1) to afford **18** (3.7 mg, 42%) as a dark violet solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.33 (bs, 1H), 9.56 (d, *J* = 9.0 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.67 (s, 1H), 7.59–7.55 (m, 2H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 9.0 Hz, 1H), 4.66 (d, *J* = 3.5 Hz, 1H), 4.22 (dq, *J* = 9.0, 6.0 Hz, 1H), 3.38 (d, *J* = 9.0 Hz, 1H), 3.19 (s, 3H), 2.71 (d, *J* = 14.7 Hz, 1H), 2.56 (s, 3H), 1.84 (dd, *J* = 14.7, 3.5 Hz, 1H), 1.67 (s, 3H), 1.43 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.1,

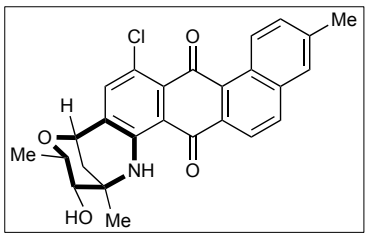
185.5, 151.1, 138.6, 137.2, 136.5, 135.3, 134.5, 134.3, 132.0, 128.9, 128.6, 128.5, 127.8, 122.9, 120.4, 116.1, 114.3, 97.7, 80.4, 64.9, 55.3, 54.9, 38.4, 26.0, 21.7, 18.6. HRMS (ESI-TOF) calcd. for $C_{27}H_{28}NO_5^+$ $[M+H]^+$ 446.1962, found: 446.1964. $[\alpha]_D^{20}$ –108 (*c* 0.0037, $CHCl_3$).



Oxamethoxymarmycin (19). To a solution of **18** (3.7 mg, 0.008 mmol) in dry dimethylformamide (2 mL) was added NaH (60% in mineral oil, 3.3 mg, 0.08 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h prior addition of methanol. The mixture was concentrated to dryness under reduced pressure and the crude residue was purified by preparative TLC (petroleum ether/ethyl acetate, 8:2) to afford **19** (2.7 mg, 75%) as a violet solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (d, *J* = 9.0 Hz, 1H), 9.40 (bs, 1H), 8.37 (d, *J* = 8.5 Hz, 1H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.66 (s, 1H), 7.60–7.55 (m, 2H), 7.06 (d, *J* = 8.5 Hz, 1H), 4.72 (d, *J* = 4.5 Hz, 1H), 3.94 (dq, *J* = 10.0, 6.0 Hz, 1H), 3.81 (dd, *J* = 10.0, 1.0 Hz, 1H), 3.28 (s, 3H), 2.55 (s, 3H), 2.28 (d, *J* = 14.5 Hz, 1H), 2.06 (dd, *J* = 14.5, 4.5 Hz, 1H), 1.38 (s, 3H), 1.31 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.0, 185.6, 145.4, 138.6, 136.7, 136.5, 135.1, 134.1, 131.9, 129.6, 129.4, 128.9, 128.7, 127.8, 122.6, 119.6, 117.7, 112.9, 97.5, 79.6, 62.1, 55.6, 48.4, 41.9, 28.5, 21.7, 17.4. HRMS (ESI-TOF) calculated for $C_{27}H_{26}NO_5^+$ $[M+H]^+$ 444.1805, found 444.1824. $[\alpha]_D^{20}$ –118 (*c* 0.0135, $CHCl_3$).

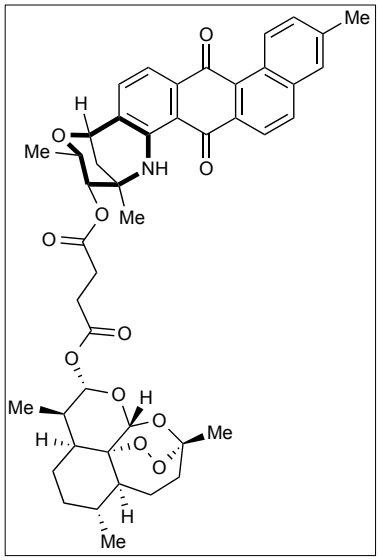


Marmycin A (1). A solution of **17** (21.0 mg, 0.043 mmol) and 50% w/w aq. HBF_4 (5 μL, 0.043 mmol) in acetonitrile (2 mL) was refluxed for 8 h. The reaction was then cooled to room temperature, diluted with dichloromethane (5 mL) and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The mixture was purified by preparative TLC (heptane/ethyl acetate, 1:1) to afford marmycin A (2.3 mg, 13%), which was recrystallized from dichloromethane:heptane to yield red needles. CCDC 1015494. ^{1}H NMR (950 MHz, $CDCl_3$) δ 9.59 (s, 1H), 9.55 (d, *J* = 9.0 Hz, 1H), 8.31 (d, *J* = 8.5 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.67 (bs, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.57 (dd, *J* = 9.0, 1.5 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 4.85–4.80 (m, 1H), 3.22 (t, *J* = 9.5 Hz, 1H), 3.17 (dq, *J* = 9.5, 6.0 Hz, 1H), 2.56 (s, 3H), 2.19 (dd, *J* = 13.0, 3.5, 1H), 1.87 (dd, *J* = 13.0, 2.0 Hz, 1H), 1.55 (s, 3H), 1.27 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (239 MHz, $CDCl_3$) δ 186.5, 185.7, 148.7, 138.8, 136.5, 136.4, 136.1, 134.7, 134.6, 132.2, 128.8, 128.5, 128.3, 127.8, 127.4, 122.3, 116.1, 111.3, 79.1, 69.3, 69.3, 51.7, 35.0, 25.0, 21.7, 18.4. HRMS (ESI-TOF) calcd. for $C_{26}H_{24}NO_4^+$ $[M+H]^+$ 414.1700, found 414.1703. $[\alpha]_D^{20}$ +333 (*c* 0.002, tetrahydrofurane).



Marmycin B (2). A solution of **1** (0.840 mg, 0.002 mmol) and *N*-chlorosuccinimide (1.6 mg, 0.0122 mmol) in dichloroethane (2 mL) was refluxed for 24 h. After this time the reaction was cooled to room temperature, diluted with dichloromethane and washed with water. The organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude residue was purified by preparative TLC using (heptane/ethyl acetate, 1:1) to afford Marmycin B (0.54 mg, 61%) as a pink solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (bs, 1H), 9.17 (d, *J* = 9.0 Hz, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 8.5 Hz, 1H), 7.68 (s, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.49 (s, 1H), 4.76 (s, 1H), 3.26–3.18 (m, 1H), 3.13 (dq, *J* = 12.0, 6.0 Hz, 1H), 2.57 (s, 3H), 2.18 (d, *J* = 10.0 Hz, 1H), 1.85 (d, *J* = 12.0 Hz, 1H), 1.54 (s, 3H), 1.24 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.5, 185.3, 148.1, 139.2, 139.1, 136.7, 133.9, 133.6, 133.1, 131.87, 131.3, 129.9, 128.1, 128.1, 127.8, 121.9, 120.9, 112.7, 79.1, 69.5, 68.9, 51.9, 35.0, 25.0,

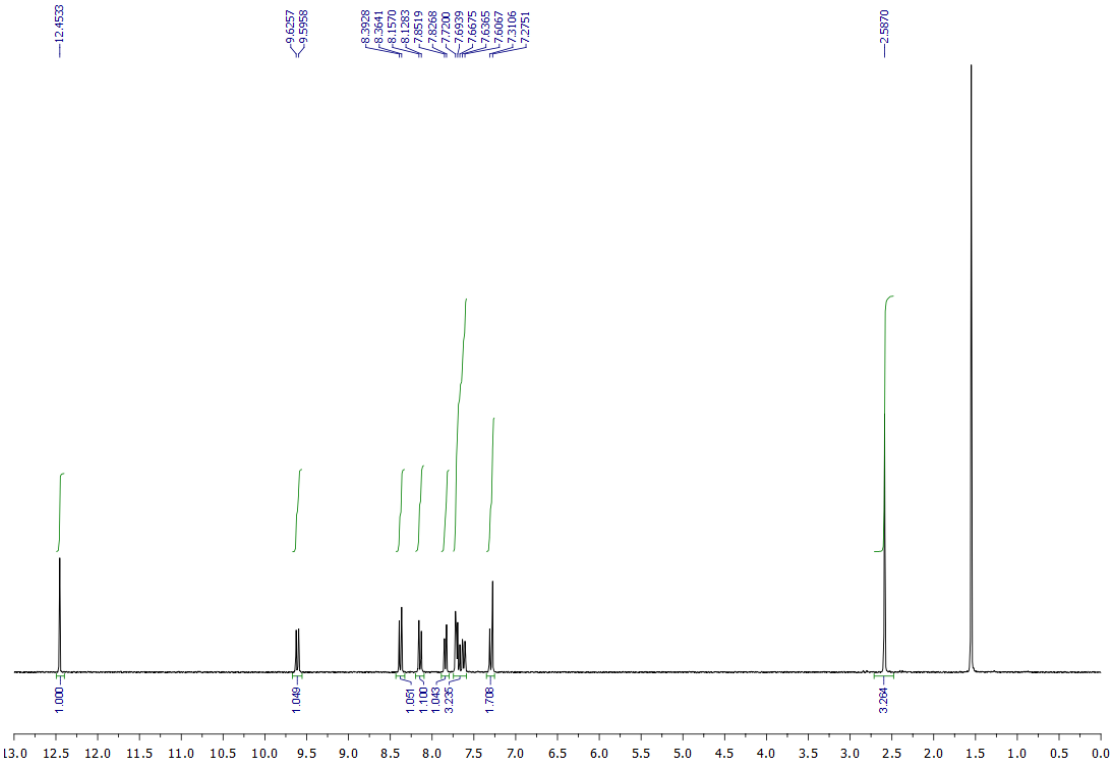
21.8, 18.4. HRMS (ESI-TOF) calcd. for $C_{26}H_{23}ClNO_4^+$ $[M+H]^+$ 448.1310, found 448.1312. $[\alpha]_D^{20}$ +132 (*c* 0.047, tetrahydrofurane).



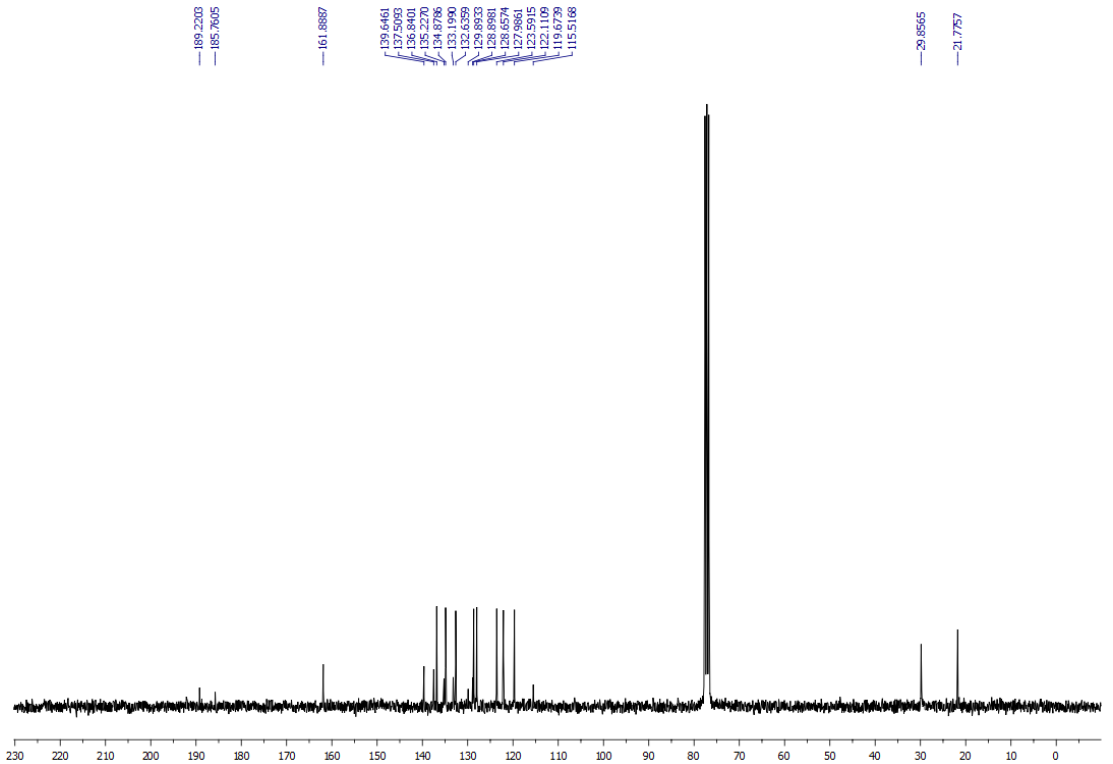
Artesumycin (21). To a mixture of **1** (2.3 mg, 0.0055 mmol), **20** (2.1 mg, 0.0056 mmol) and DMAP (0.07 mg, 0.0006 mmol, 10 mol%) in dry dichloromethane (0.3 mL) was added a solution of N,N'-dicyclohexylcarbodiimide (1.1 mg, 0.0055 mmol) in dichloromethane (0.2 mL) at 0° C. The reaction mixture was stirred at room temperature overnight and was then concentrated to dryness under reduced pressure. The crude product was purified by preparative TLC (heptane/AcOEt, 1:1) to afford **21** (1.8 mg, 42%) as a red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (s, 1H), 9.59 (d, *J* = 9.0 Hz, 1H), 8.39 (d, *J* = 8.5 Hz, 1H), 8.11 (d, *J* = 9.0 Hz, 1H), 7.69 (s, 1H), 7.63–7.56 (m, 2H), 7.50 (d, *J* = 7.5 Hz, 1H), 5.80 (d, *J* = 9.5 Hz, 1H), 5.41 (s, 1H), 4.83 (s, 1H), 4.79 (d, *J* = 9.5 Hz, 1H), 3.44 (dq, *J* = 12.0, 6.0 Hz, 1H), 2.79–2.74 (m, 4H), 2.58–2.54 (m, 4H), 2.36 (td, *J* = 14.0, 4.0 Hz, 1H), 2.25 (dd, *J* = 13.0, 2.0 Hz, 1H), 2.02 (ddd, *J* = 7.5, 4.0, 3.0 Hz, 1H), 1.89–1.86 (m, 2H), 1.75 (dd, *J* = 13.5, 4.0 Hz, 2H), 1.67 (dd, *J* = 13.0, 3.0 Hz, 1H), 1.62–1.58 (m, 2H), 1.44-1.41 (m, 4H), 1.42 (s, 3H), 1.35 (dd, *J* = 13.0, 3.0 Hz, 1H), 1.09 (d, *J* = 6.0 Hz, 3H), 1.02–0.95 (m, 1H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.6, 185.6, 172.1, 171.2, 148.6, 138.7, 136.5, 135.9, 134.9, 134.6, 132.2, 128.8, 128.6, 128.3, 127.8, 126.7, 122.5, 115.9, 111.0, 104.6, 92.3, 91.5, 80.2, 79.6, 69.3, 66.4, 51.5, 51.1, 45.2, 37.3, 36.2, 35.0, 34.1, 31.9, 29.8, 29.1, 28.9, 26.1, 25.1, 24.6, 22.1, 21.7, 20.3, 18.0, 12.2. HRMS (ESI-TOF) calcd. for $C_{45}H_{49}NNaO_{11}^+$ $[M+Na]^+$ 802.3198, found 802.3204. $[\alpha]_D^{20}$ +253 (*c* 0.015, tetrahydrofurane).

NMR and mass spectra

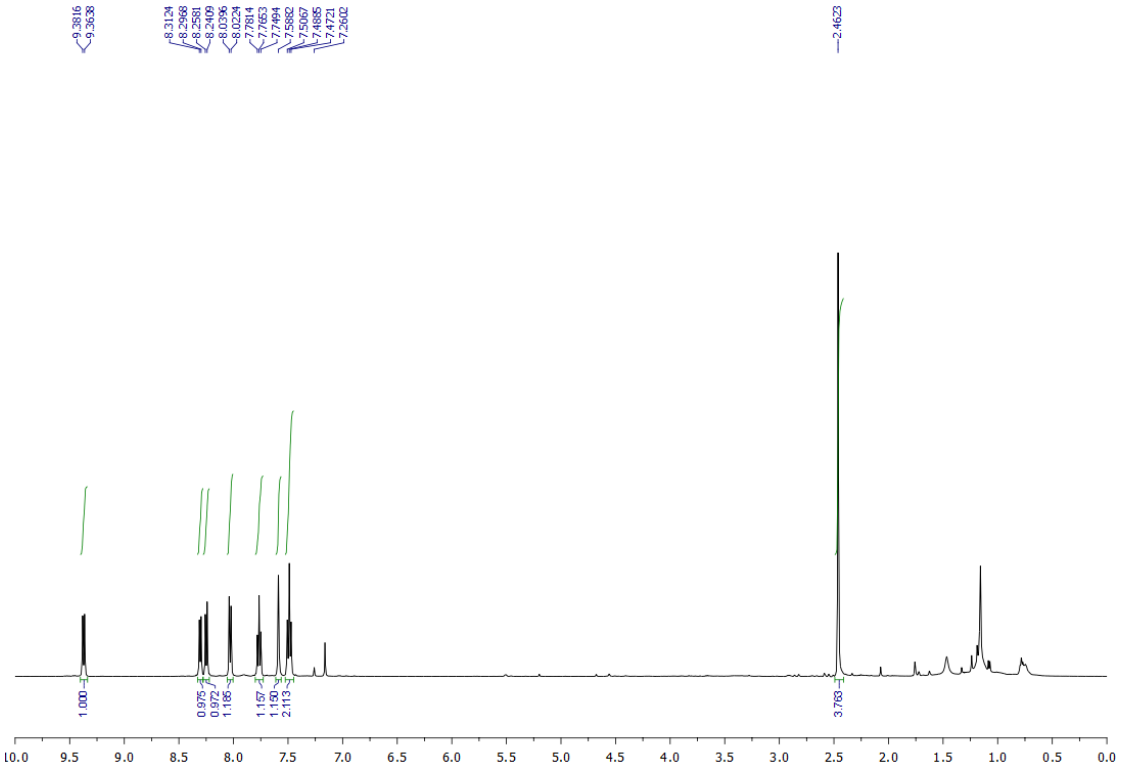
^{1}H NMR spectrum of **14** in $CDCl_3$



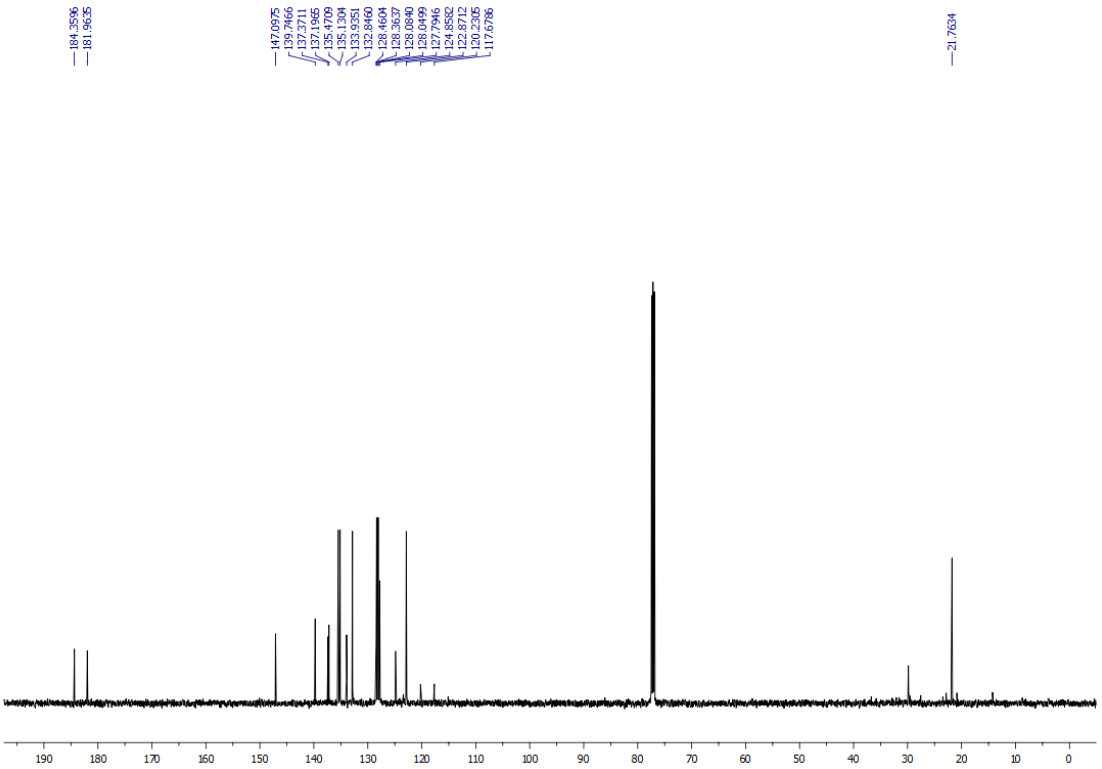
^{13}C NMR spectrum of **14** in $CDCl_3$



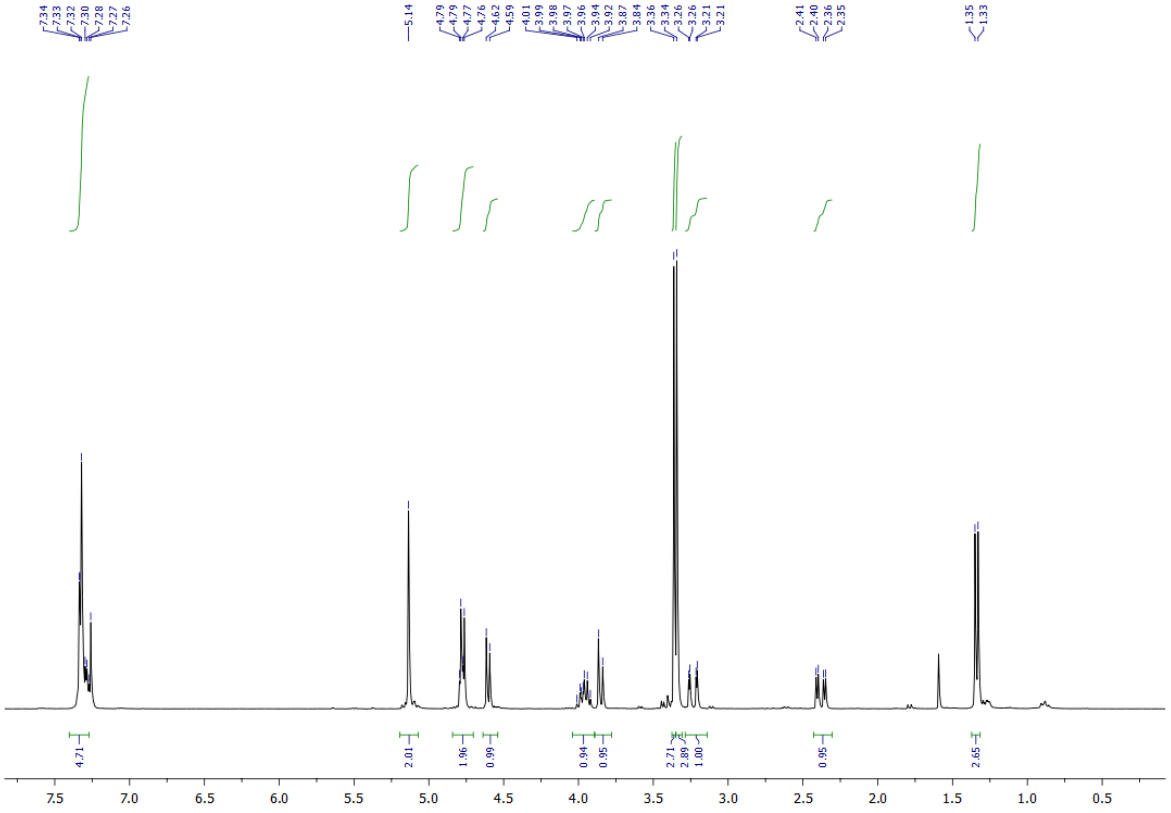
^{1}H NMR spectrum of **7** in $CDCl_3$



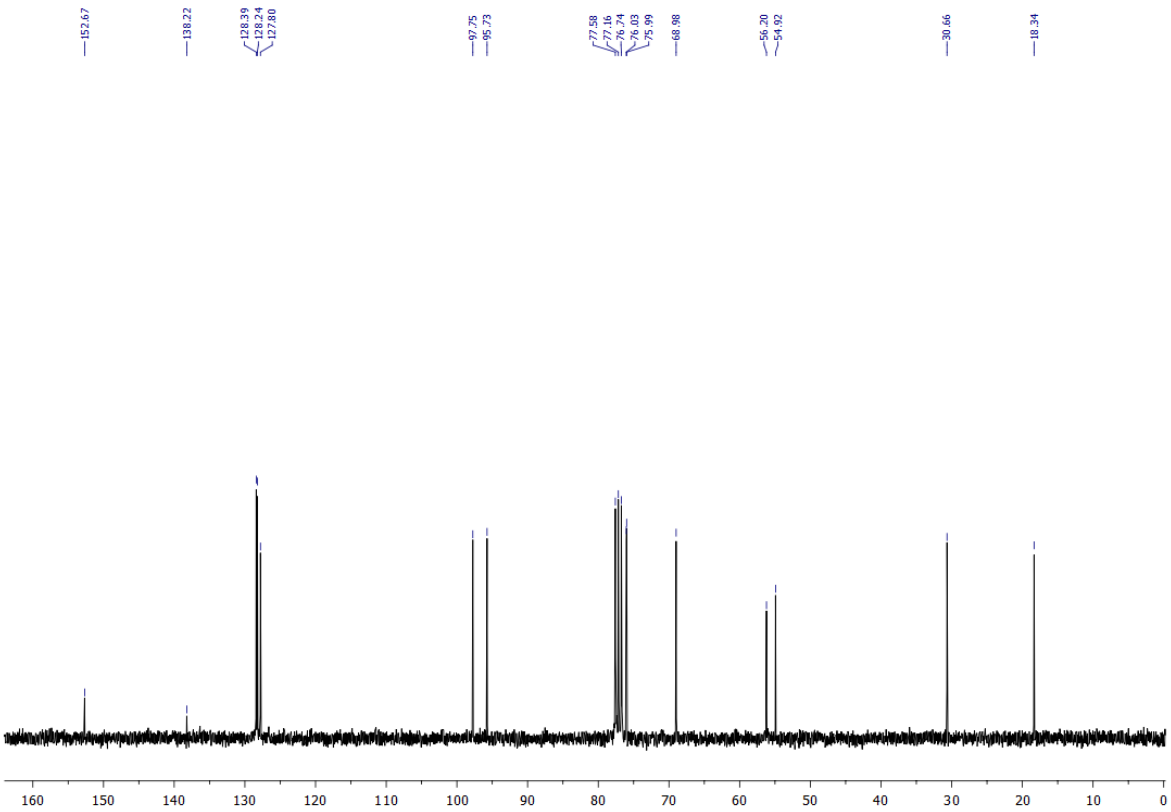
^{13}C NMR spectrum of **7** in $CDCl_3$



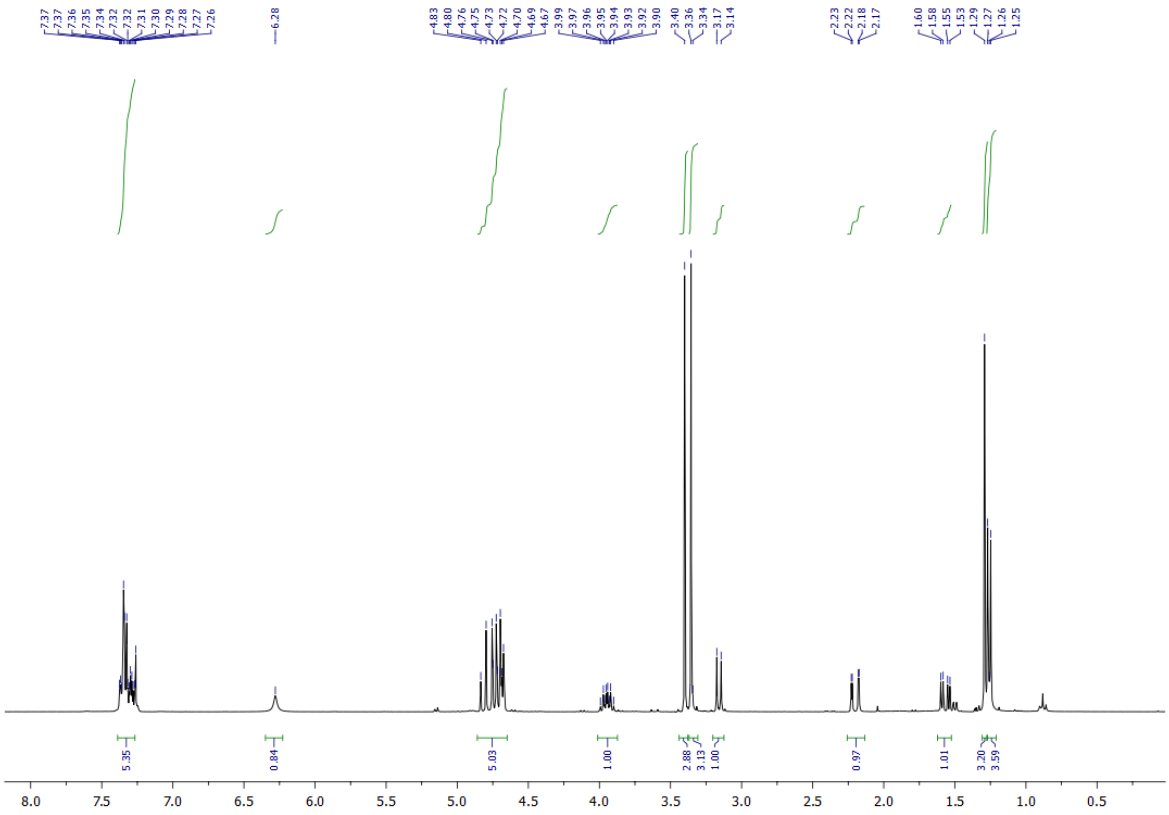
^{1}H NMR spectrum of **16** in $CDCl_3$



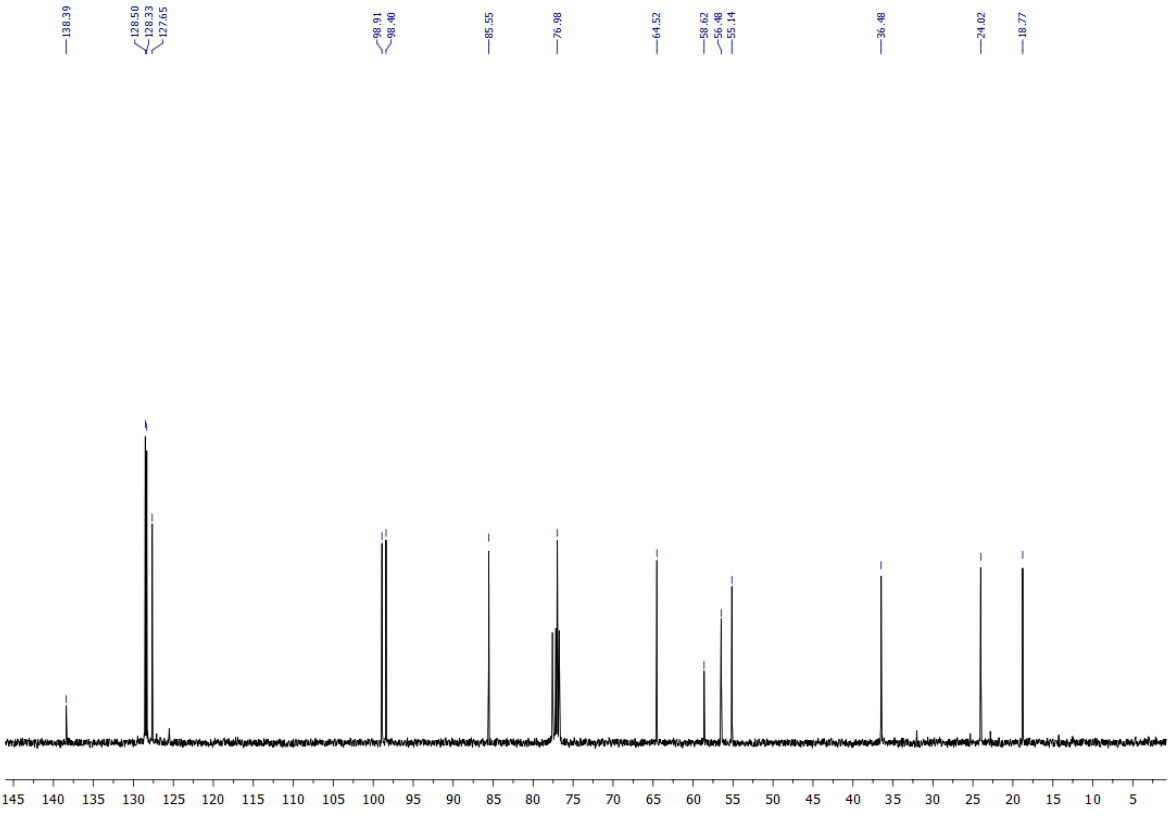
^{13}C NMR spectrum of **16** in $CDCl_3$



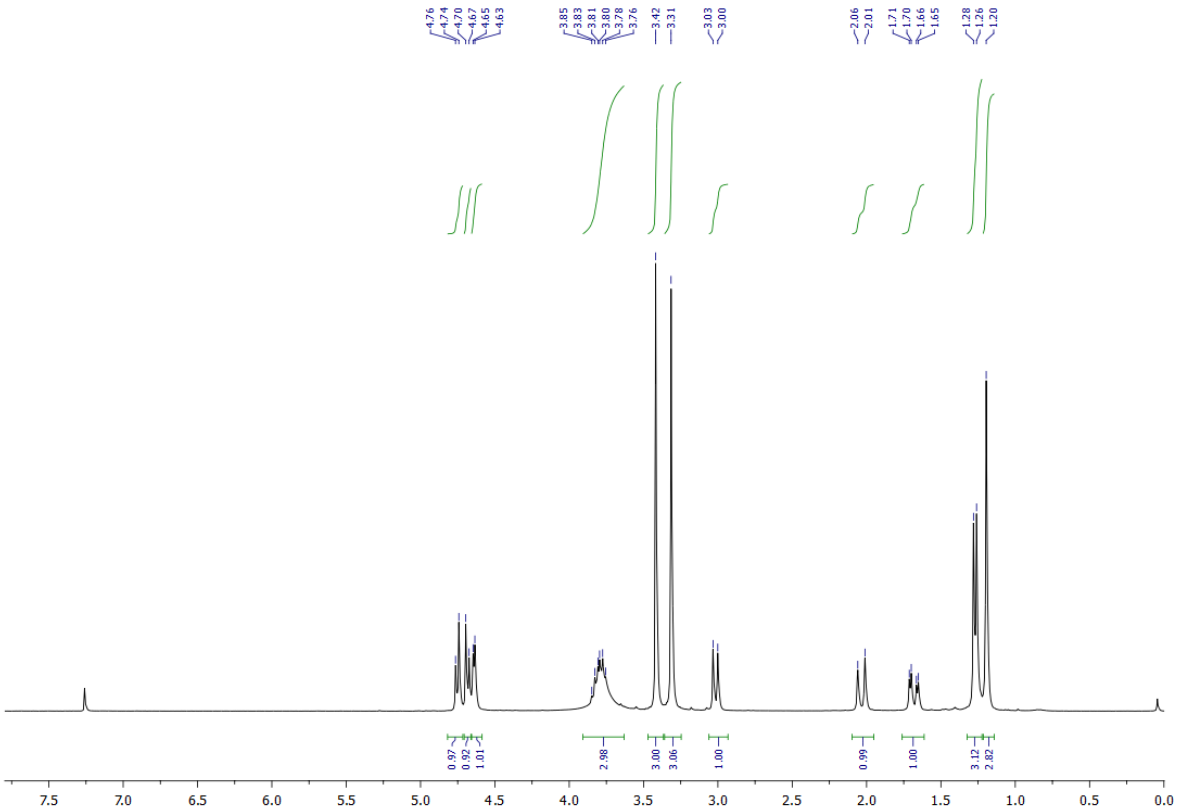
^{1}H NMR spectrum of **29** in $CDCl_3$



^{13}C NMR spectrum of **29** in $CDCl_3$



^{1}H NMR spectrum of **8** in $CDCl_3$



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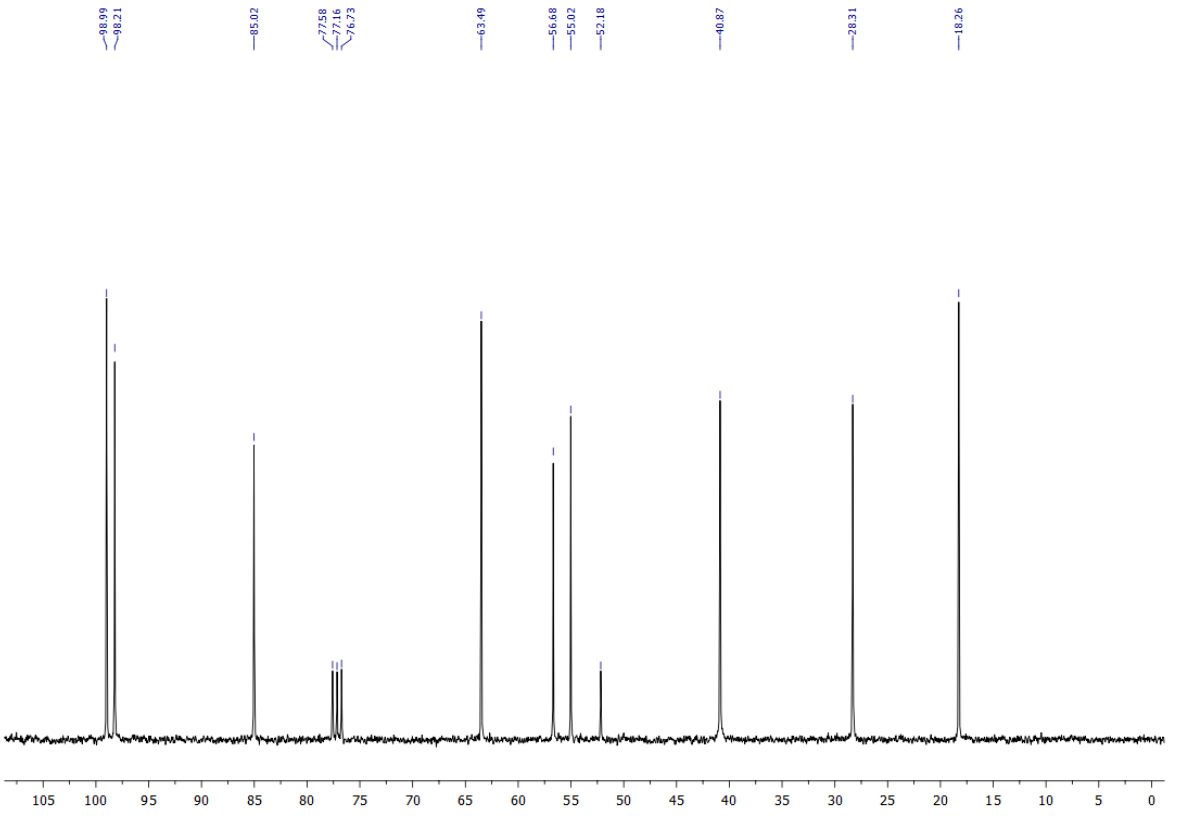
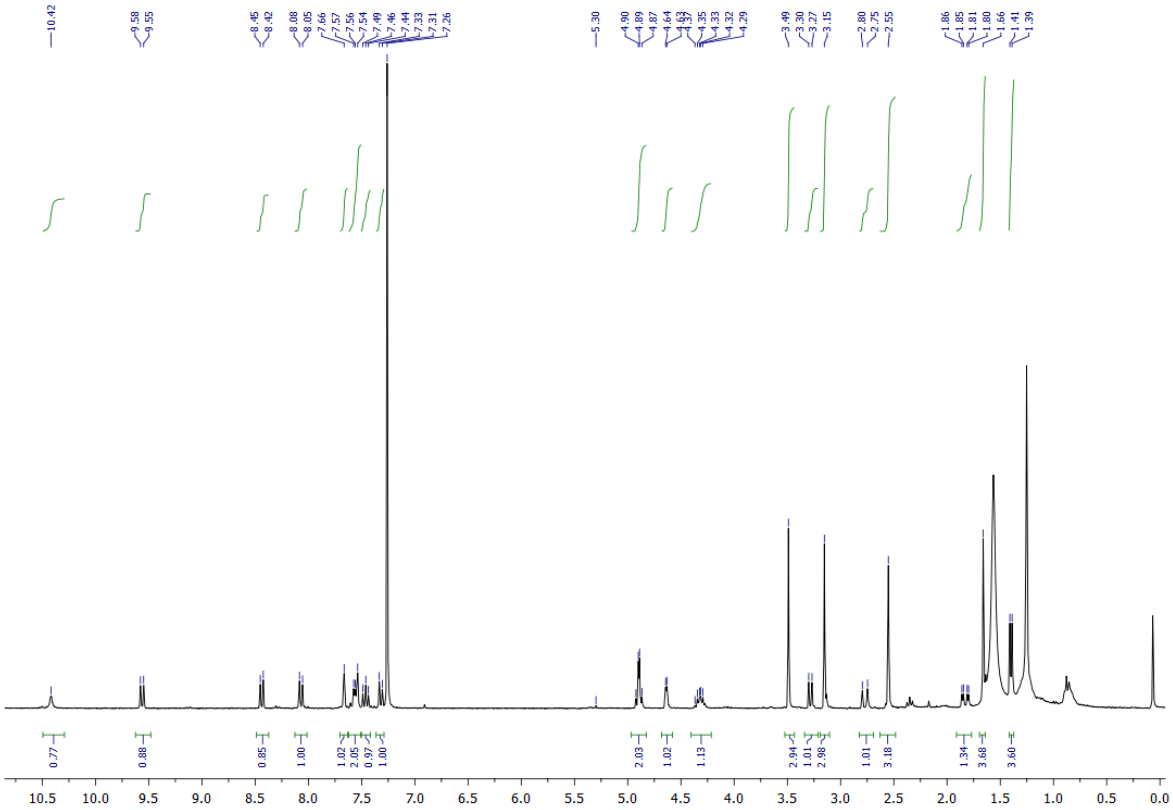
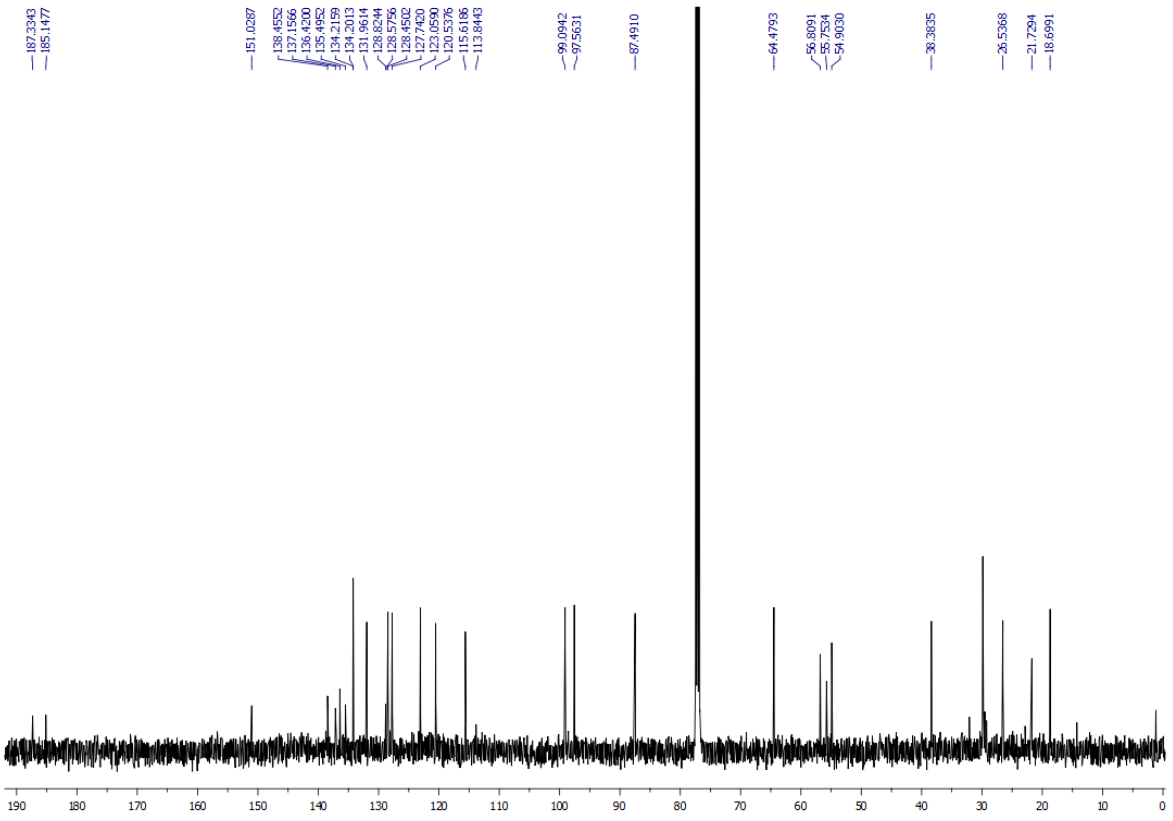


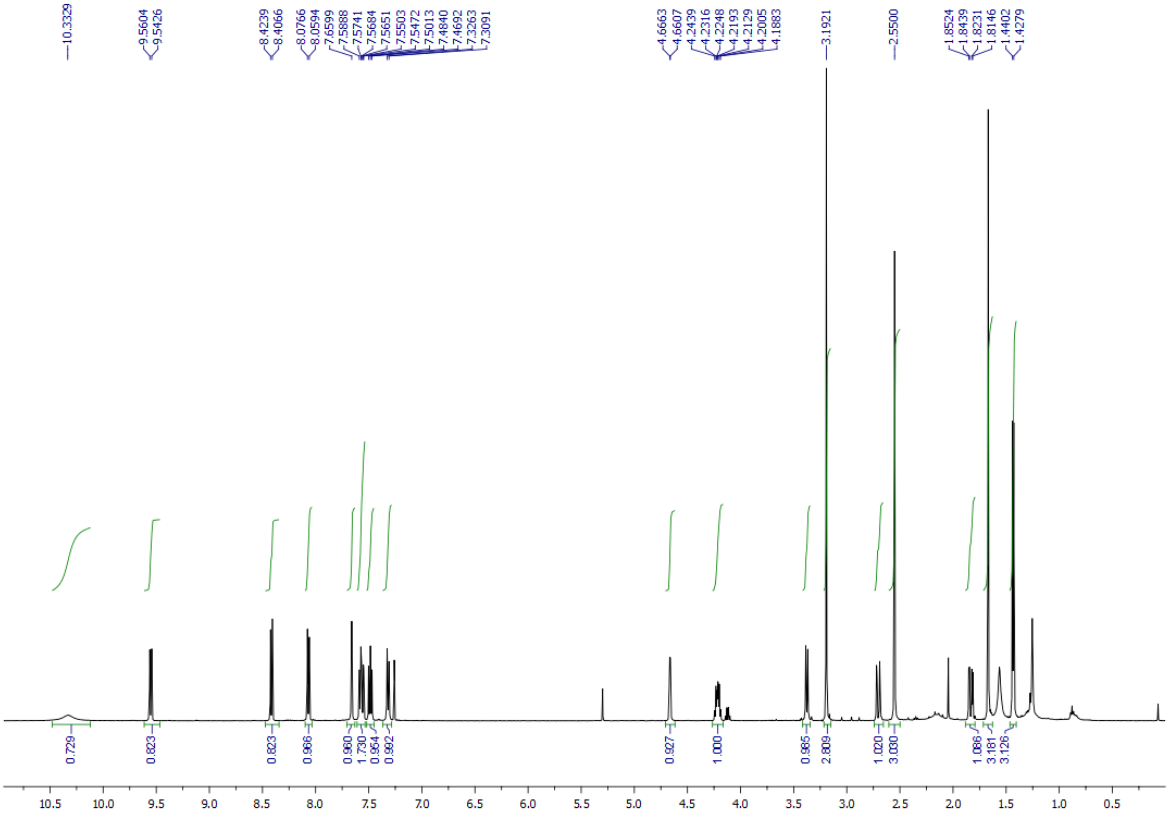
^{1}H NMR spectrum of **17** in $CDCl_3$



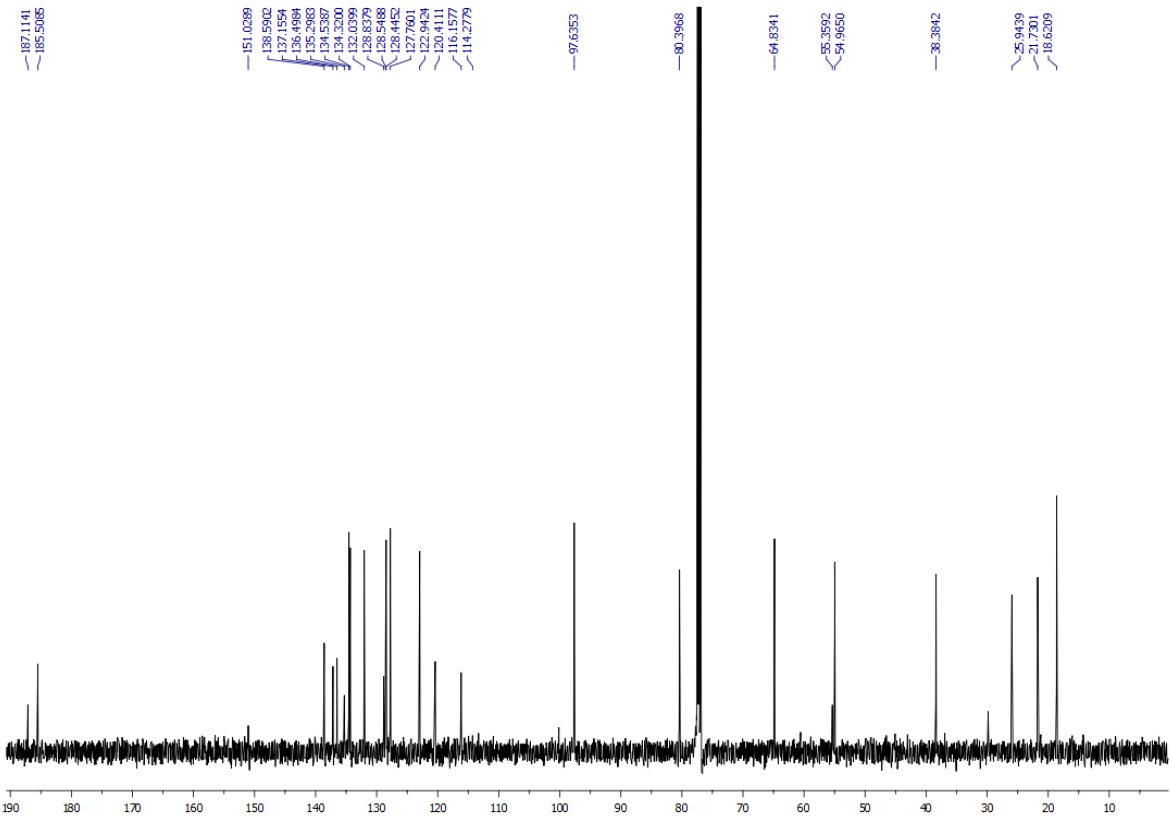
^{13}C NMR spectrum of **17** in $CDCl_3$



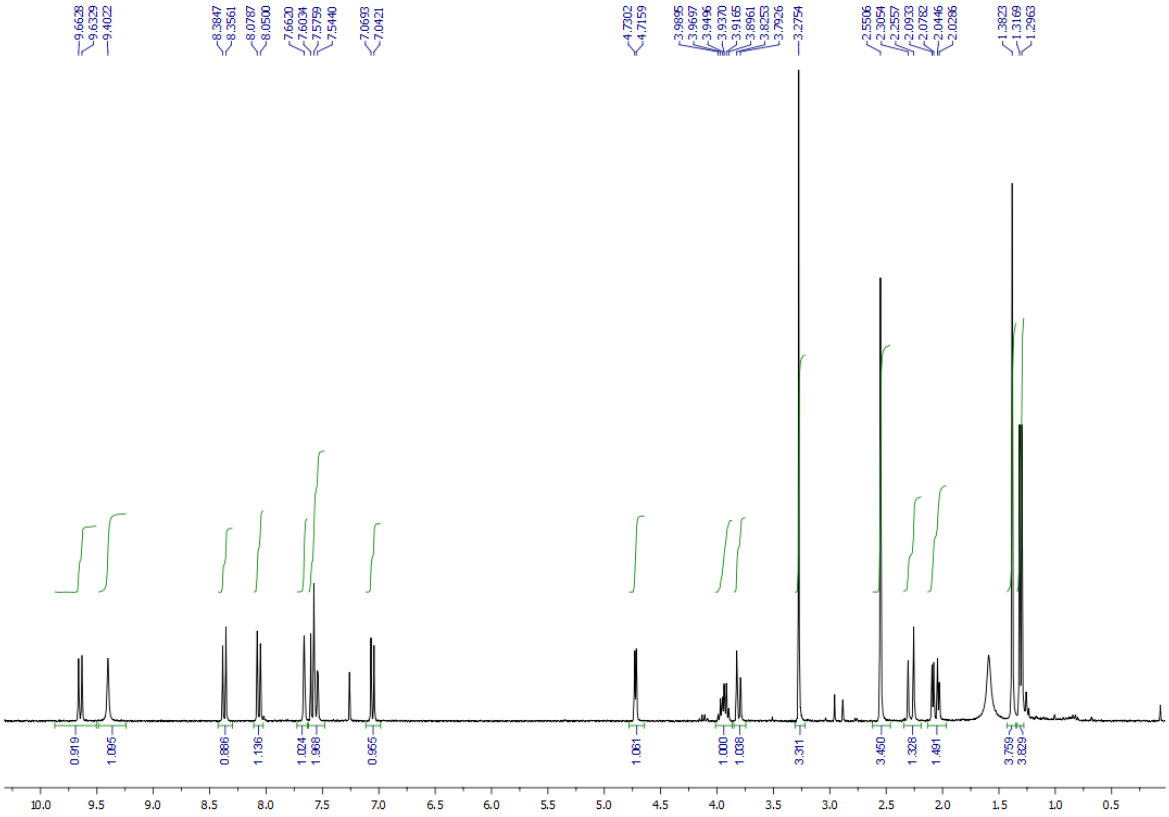
^{1}H NMR spectrum of **18** in $CDCl_3$



^{13}C NMR spectrum of **18** in $CDCl_3$



^{1}H NMR spectrum of **19** in $CDCl_3$



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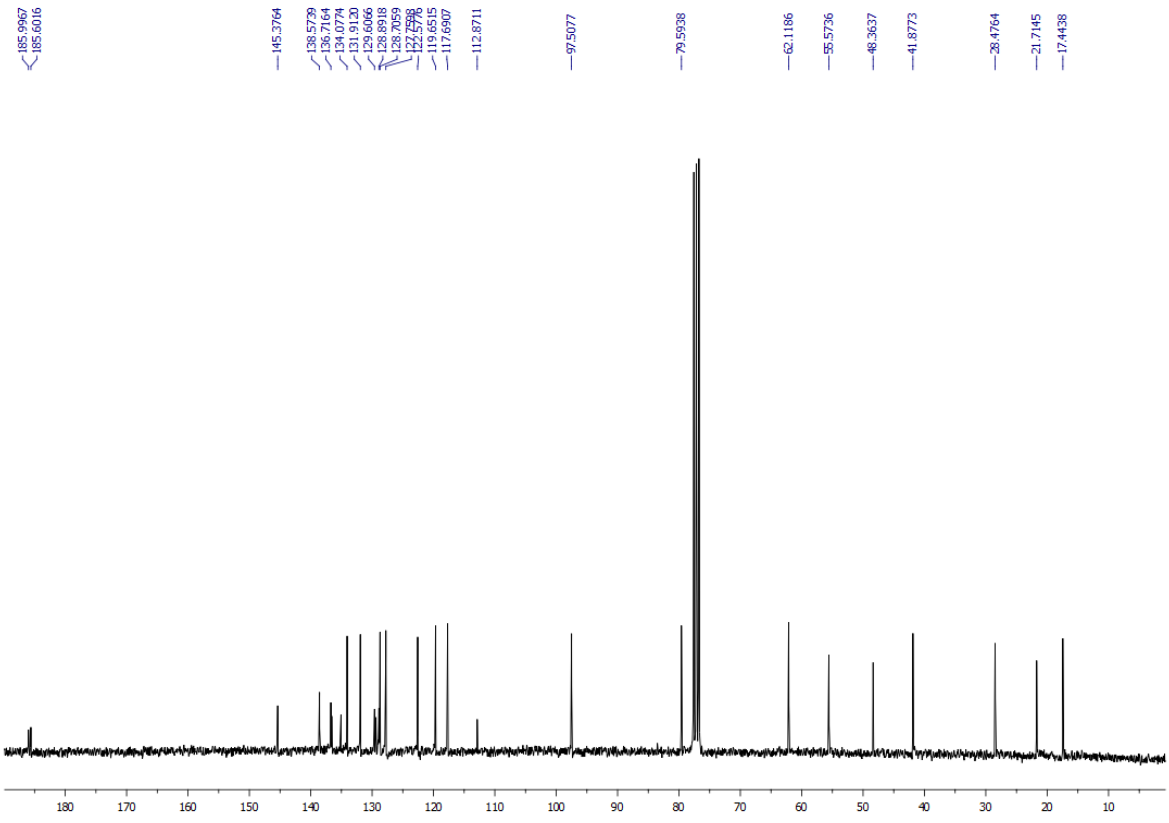

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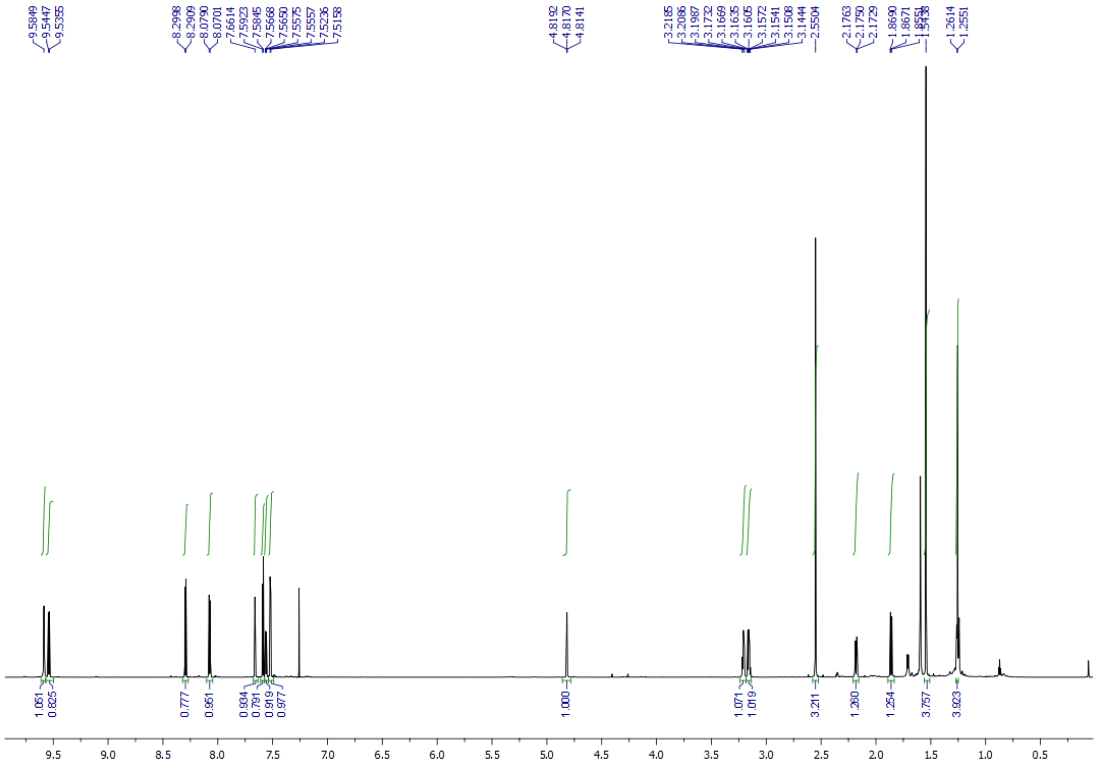
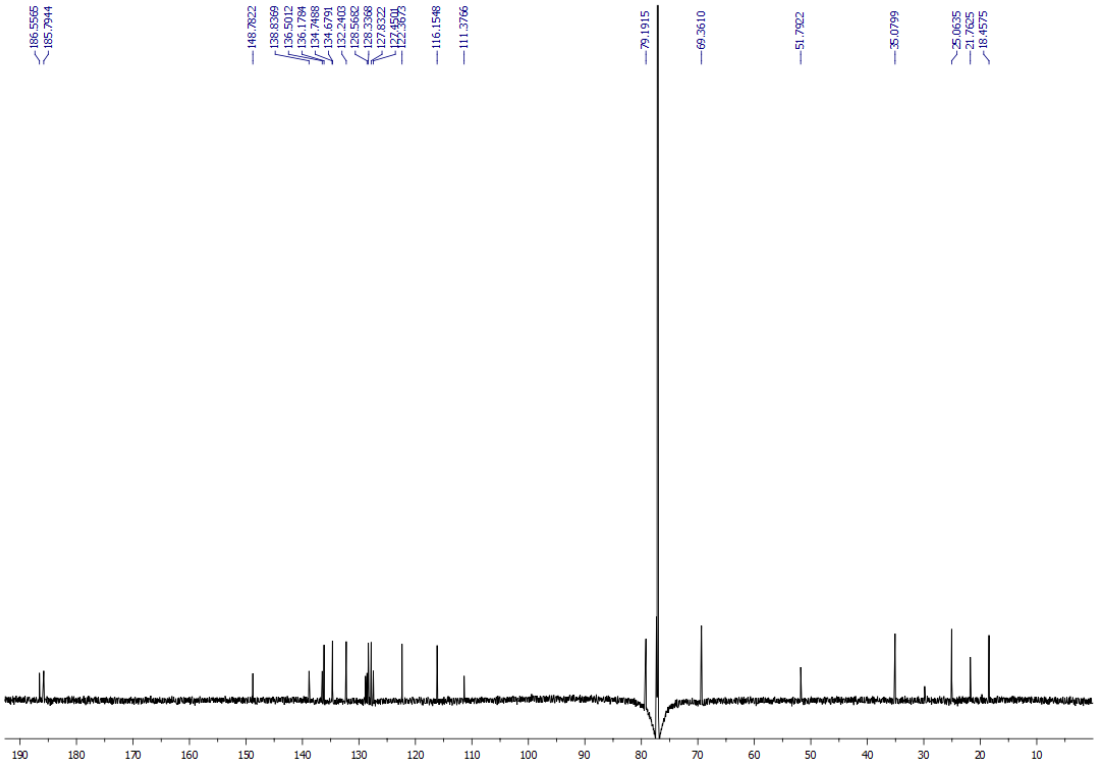
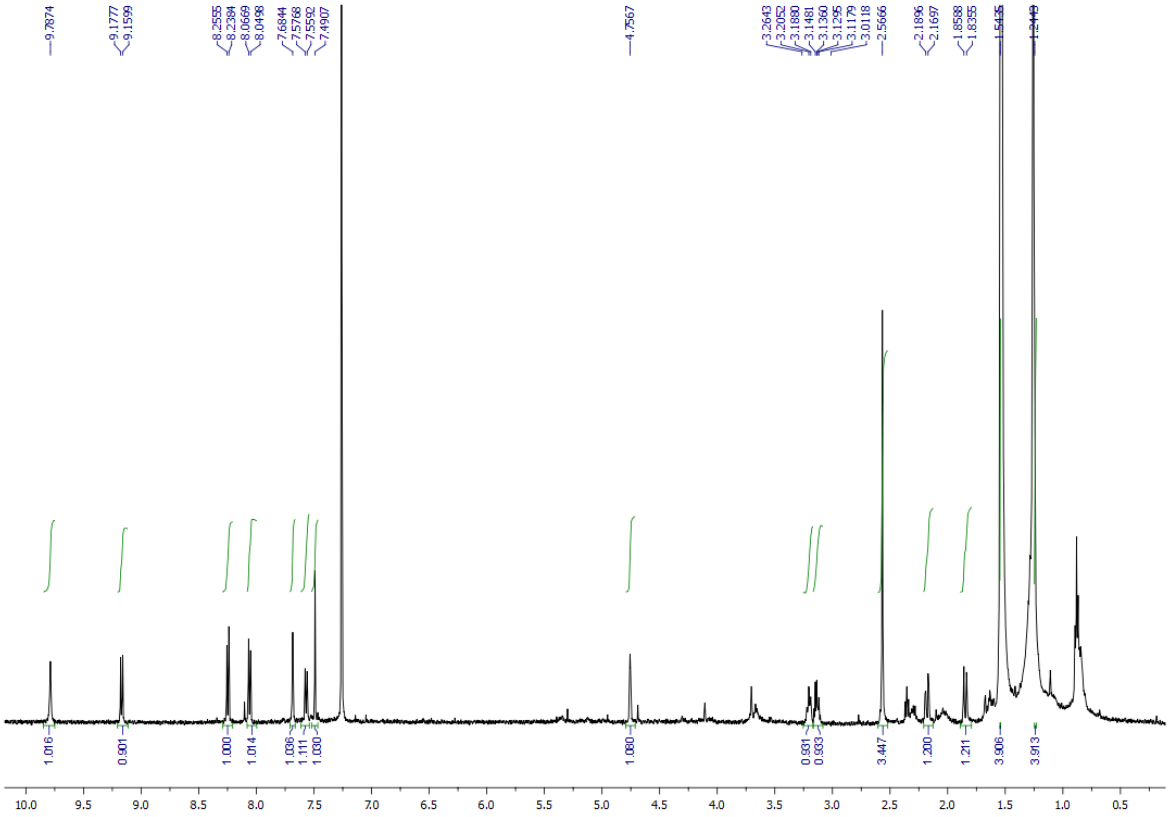


^{13}C NMR spectrum of **1** in $CDCl_3$



^{1}H NMR spectrum of **2** in $CDCl_3$



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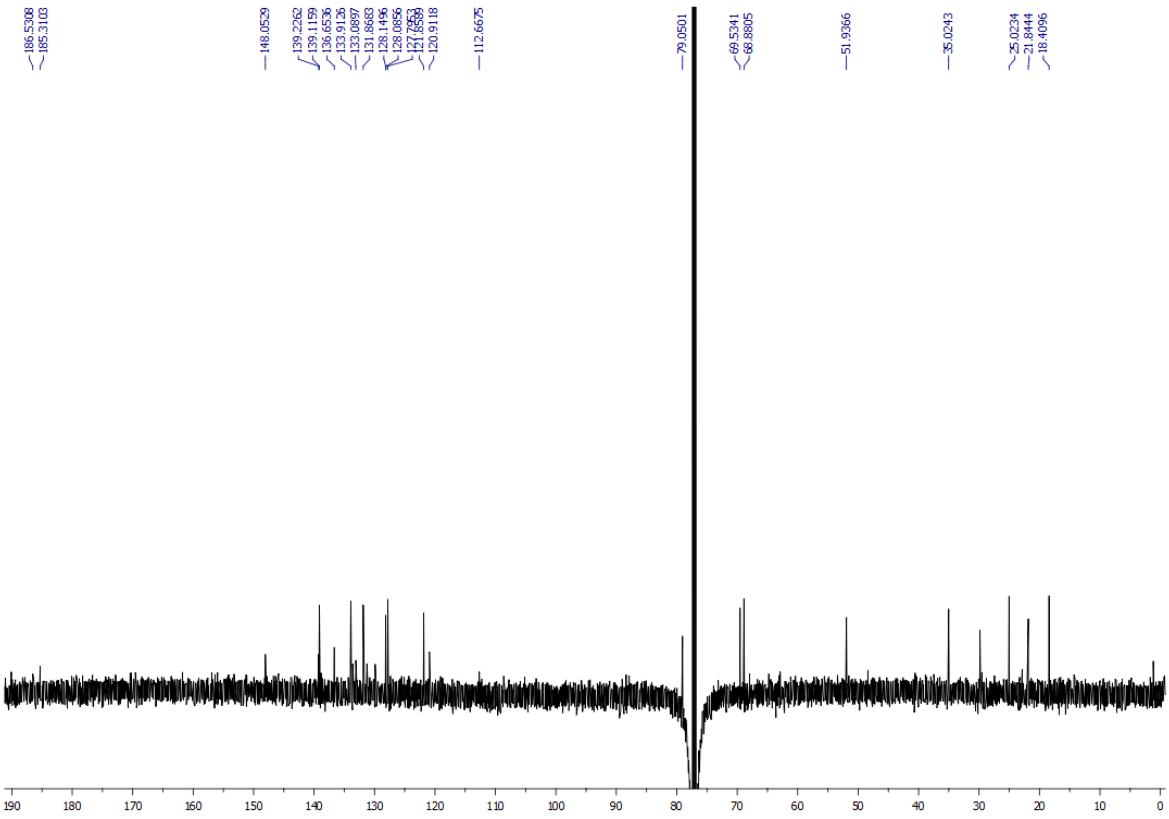
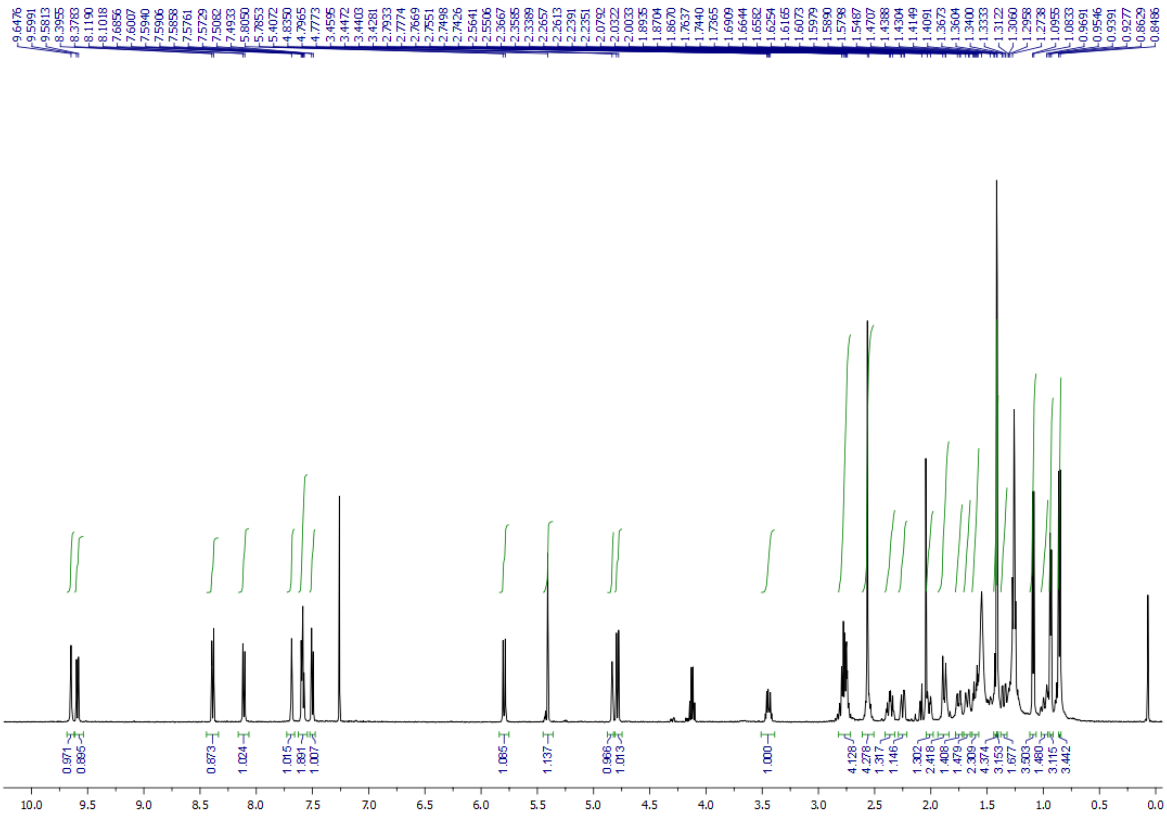


^{1}H NMR spectrum of **21** in $CDCl_3$



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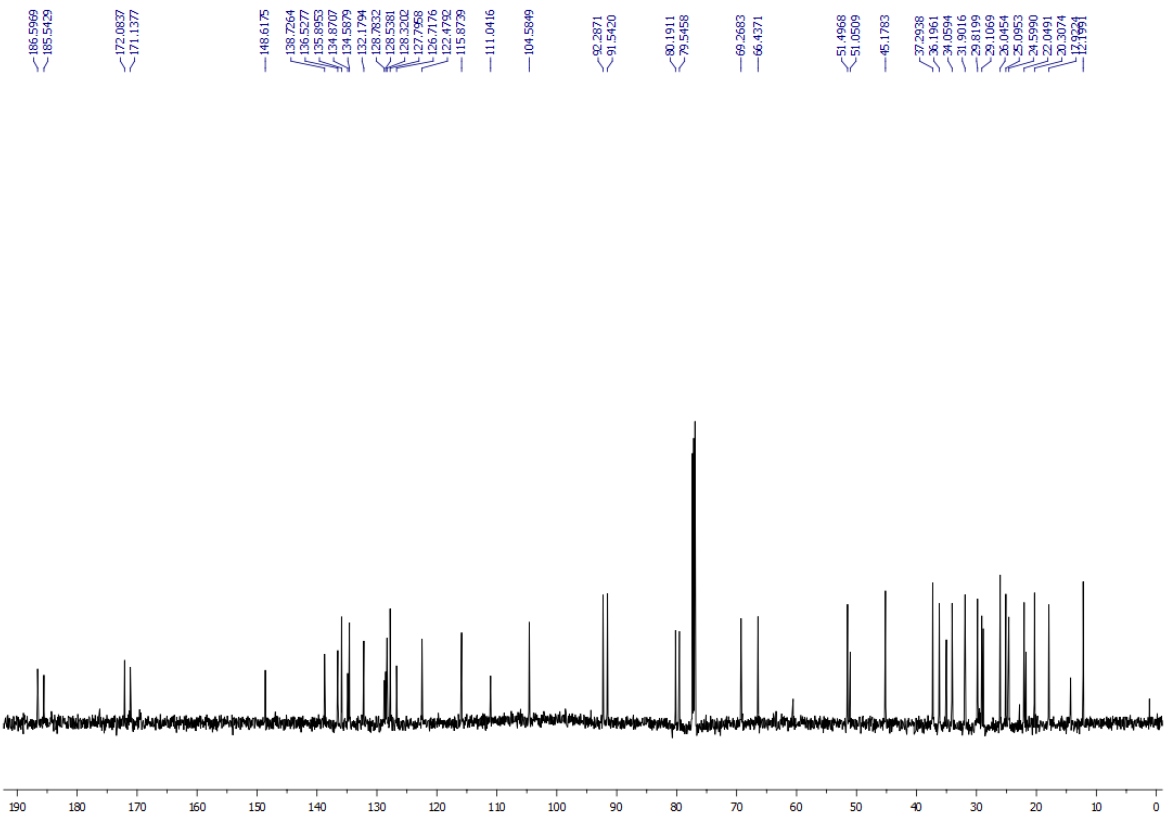
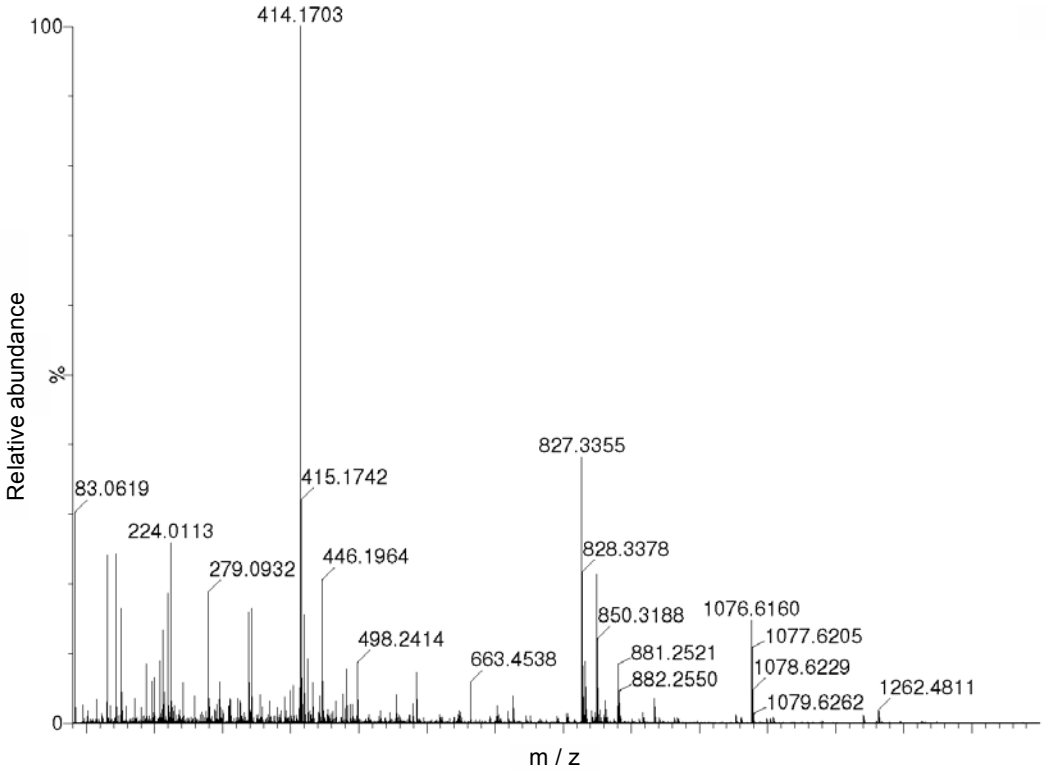


HRMS of **1**



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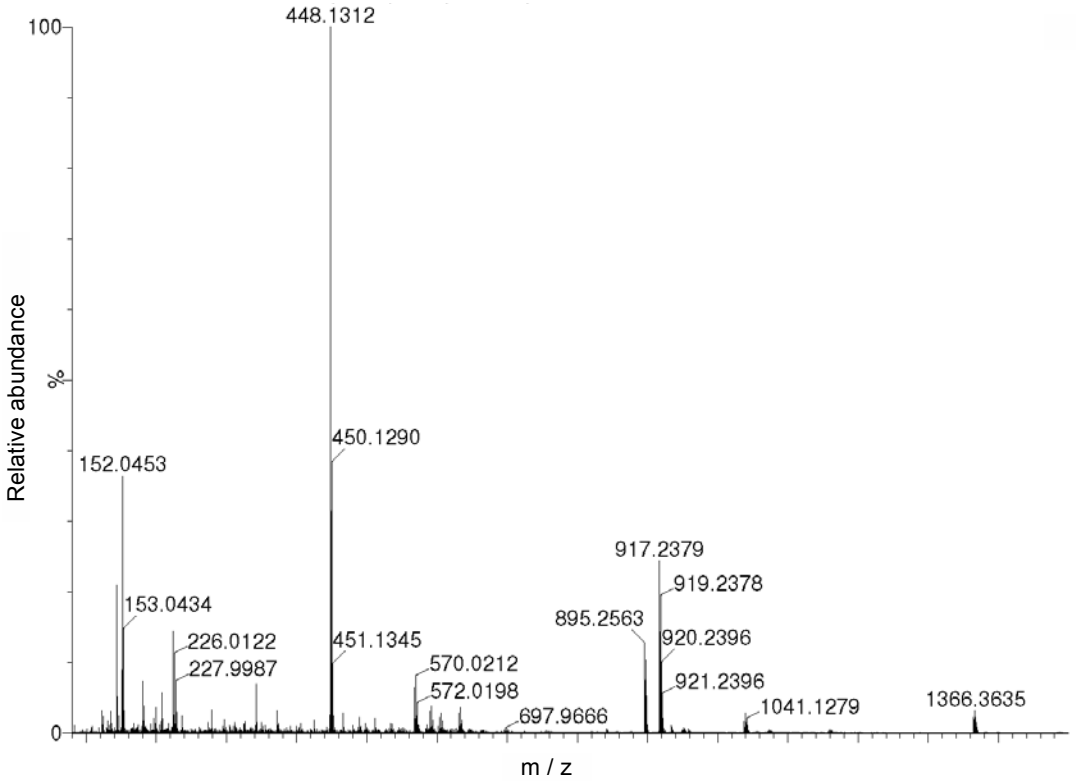
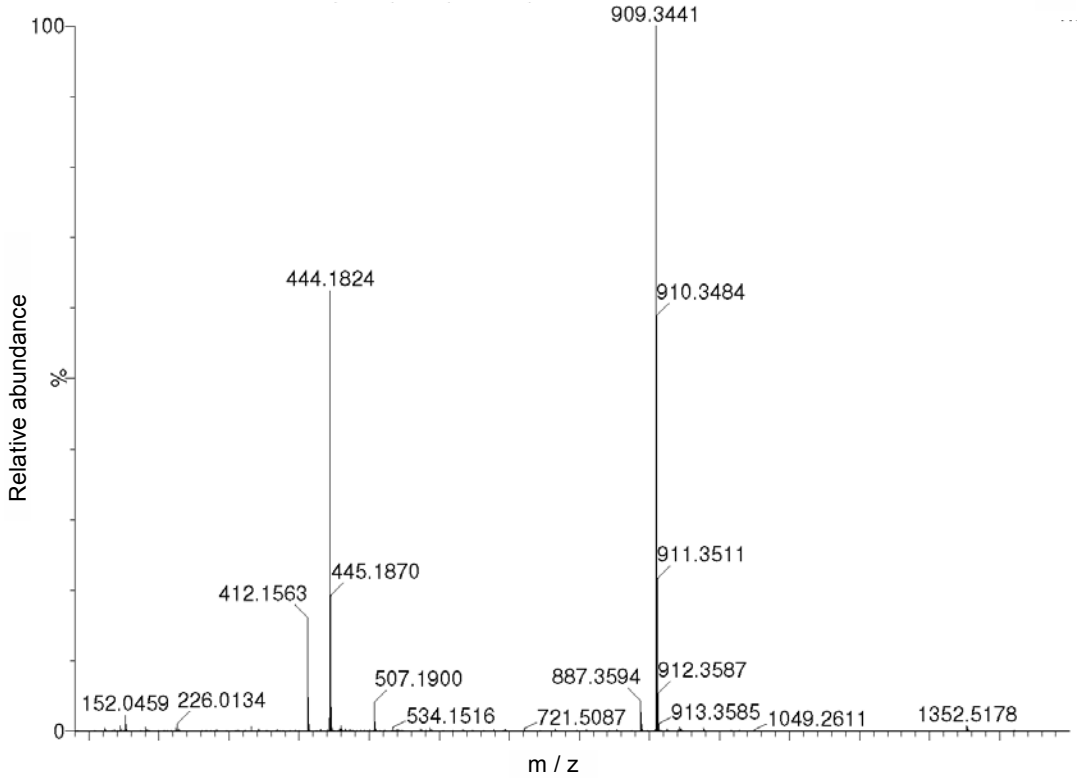
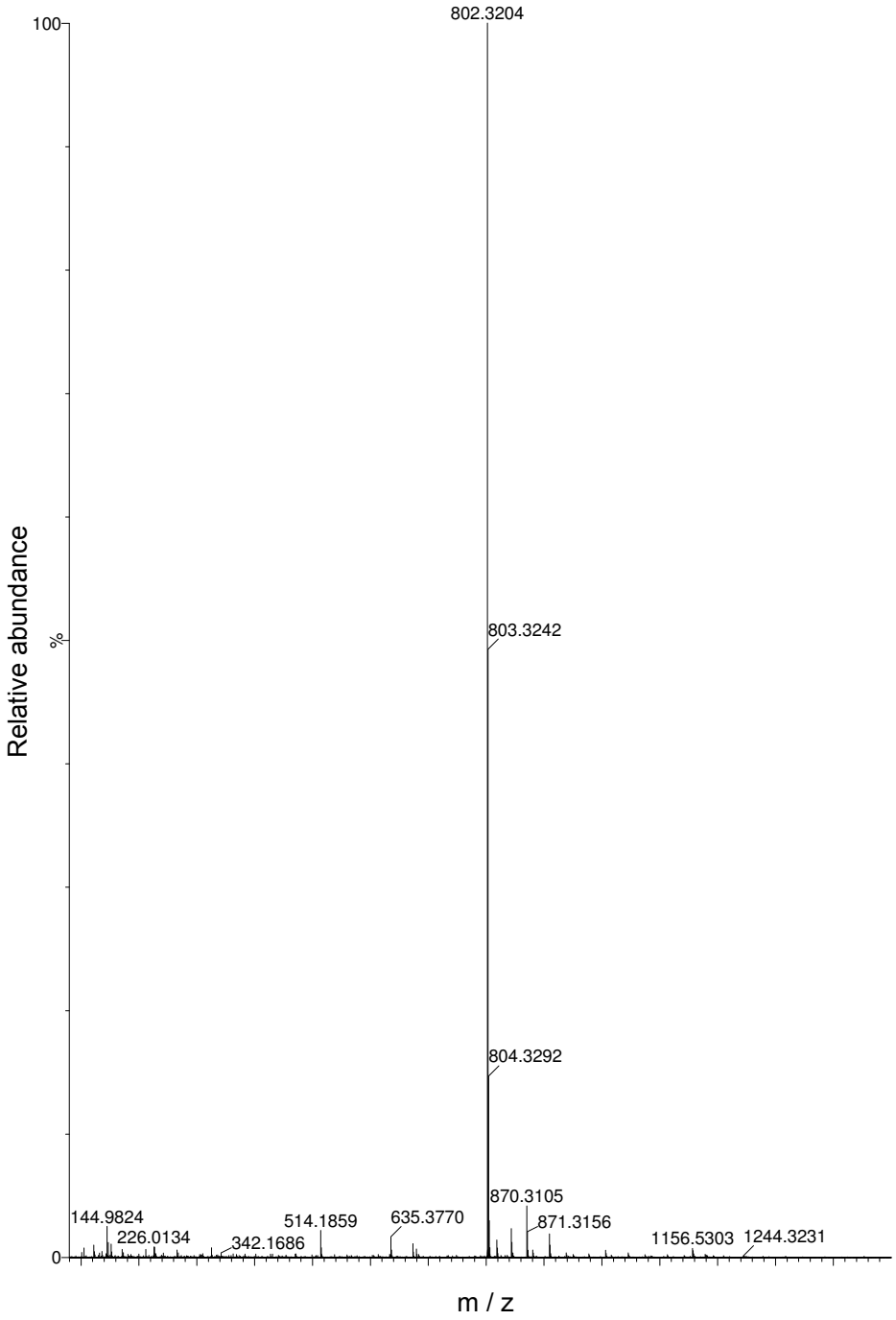


HRMS of **19**



HRMS of **21**



Crystallographic data

Bond precision: C-C = 0.0093 A Wavelength=1.54187

Cell: a=7.0956(2) b=11.5517(3) c=24.1710(17)
alpha=90 beta=90 gamma=90

Temperature: 293 K

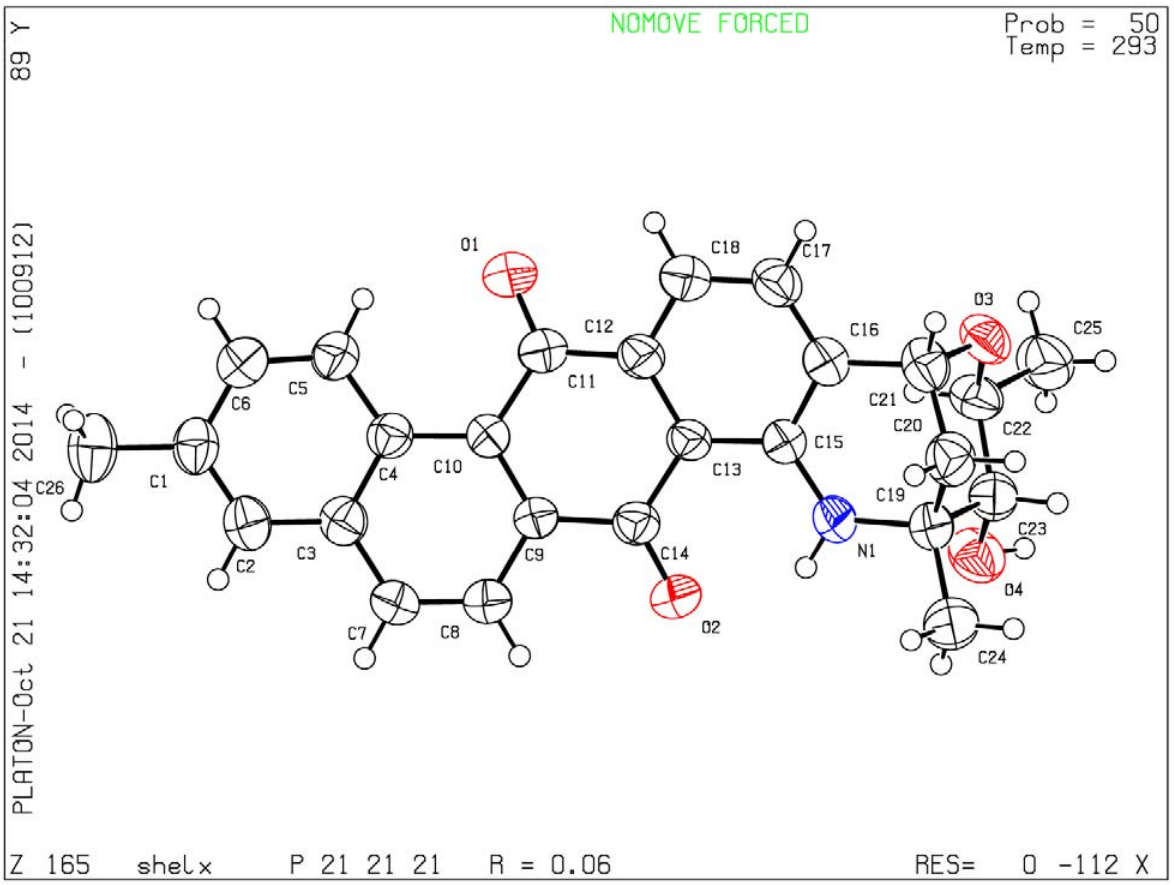
	Calculated	Reported
Volume	1981.21(16)	1981.21(16)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C26 H23 N O4	C26 H23 N O4
Sum formula	C26 H23 N O4	C26 H23 N O4
Mr	413.45	413.45
Dx,g cm-3	1.386	1.386
Z	4	4
Mu (mm-1)	0.755	0.755
F000	872.0	872.0
F000'	874.68	
h,k,lmax	8,14,29	8,14,29
Nref	3902[2256]	3793
Tmin,Tmax	0.870,0.956	0.711,1.000
Tmin'	0.857	

Correction method= MULTI-SCAN

Data completeness= 1.68/0.97 Theta(max)= 72.139

R(reflections)= 0.0593(1748) wR2(reflections)= 0.2050(3793)

S = 1.018 Npar= 288



2. Cell and Molecular Biology

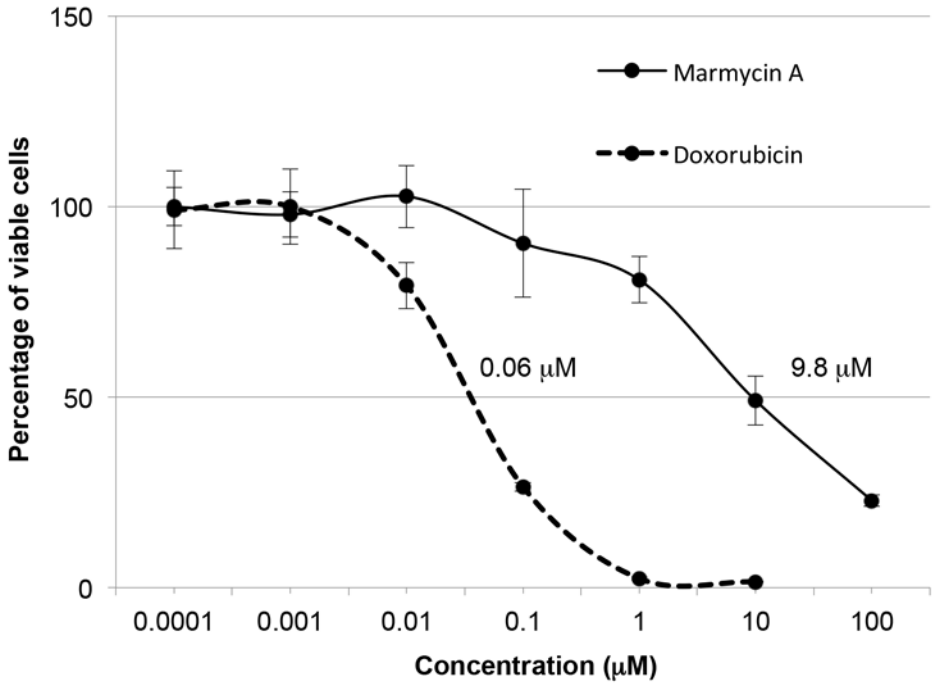
Cell culture and proliferation assays. U2OS, A2780 and MDA-MB-231 cells were purchased from ATCC and were maintained in McCoy's 5A or RPMI 1640, respectively, supplemented with 10% fetal bovine serum (FBS) and 1× Antibiotic-Antimycotic (Gibco®) and incubated at 37 °C with 5% CO_2. Cell viability assays were carried out by plating 2,000 cells per well in 96-well plates. Cells were treated with the relevant drug for 24 h or 72 h, then incubated with CellTiter-Blue® (20µL/well) for 1 h before recording fluorescence (560(20)Ex/590(10)Em) using a PerkinElmer Wallac 1420 Victor^2 Microplate Reader. For cell cycle analysis, cells were fixed in ice-cold 70% ethanol, stained with propidium iodide (10 mg/ml PI, 250 mg/ml RNase A, 0.5% BSA, 0.02% sodium azide in 1× PBS) and analyzed by FACS on a CyFlow Ploidy Analyser from Partec.

Drugs and inhibitors. Marmycin A was prepared in the laboratory according to Fig. 2. Cells were treated with 10 µM marmycin A for 24 h unless stated otherwise. Doxorubicin (Sigma) was used at 1 µM for 6 h (cell imaging) or 1 µM for 24 h (western blotting); chloroquine (Sigma) was used at 100 µM for 24 h; *N*-acetyl cysteine (Sigma) was used at 2 mM for 24 h (2 h pre-treatment); Z-DEVD-FMK (BD Biosciences) was used at 100 µM for 24 h (30 min pre-treatment); Z-VAD-FMK (BD Biosciences) was used at 100 µM for 24 h (30 min pre-treatment); E-64 (Sigma) was used at 15 µM (added 48 h after **1**, cells harvested at 96 h); CA-074 Me (Enzo Life Sciences) was used at 1 µM (added 48 h after **1**, cells harvested at 96 h); *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK, Sigma) was used at 5 µM (added 48 h after **1**, cells harvested at 96 h). Artesunate was purchased from Sigma.

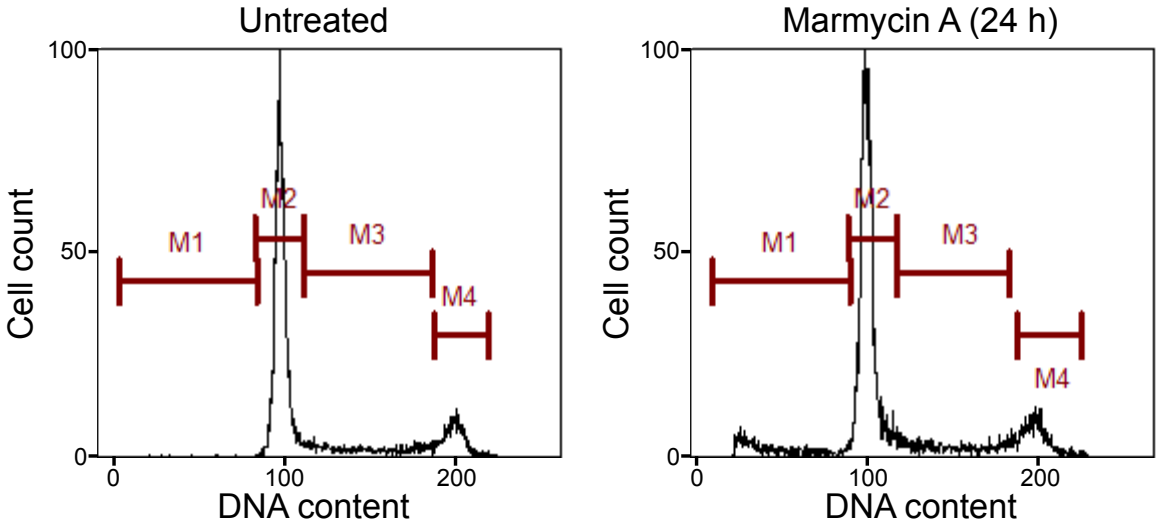
Immunofluorescence analysis and microscopy. U2OS cells cultured and treated with **1** or DXR at ~40% confluence. LysoTracker® Blue DND-22 (L7525, Life Technologies) and **1** were added and used without fixation in live cell imaging experiments. Fluorescence was recorded 10 min after addition of both reagents. GFP-Lamp1 was transiently expressed in U2OS cells following the manufacturer instructions. Briefly, 5 mL of CellLight® Lysosomes-GFP BacMam 2.0 (C10596, Life Technologies) was mixed well with 200 µM U2OS culture medium and added to each well of a 24-well plate loaded poly-L-lysine–treated coverslips. After 16 h incubation, cells were washed with PBS and treated with **1** (10 µM, 6h). Cells were then washed with PBS (phosphate buffered saline), fixed for 12 min with 2% formaldehyde/PBS and permeabilized for 10 min with 0.1% Triton X-100/PBS, then washed with PBS. The golgi apparatus was detected using an anti-RCAS1 antibody (1/100, #12290, Cell Signaling) and anti-rabbit Alexa Fluor® secondary antibody (1/500, A-11008, Life Technologies). Coverslips were mounted with VectaShield® mounting medium with or without DAPI (Vector Laboratories Ltd). Lysosomal membrane permeabilization was assessed by monitoring the release of FITC-Dextran from lysosomes[7]. In brief, cells were incubated with 1 mg/ml of FITC-Dextran (wt 10,000; FD10S, Sigma) for 2 h at 37 °C. Cells were washed, chased with culture medium for 2 h and remained either untreated (DMSO control) or were treated with marmycin A (10 µM, 96h). Cells were fixed with ice-cold methanol and analyzed by fluorescence microscopy. Images were taken with Leica SP8 microscope or Nikon Te(-E) inverted confocal microscopes equipped with a spinning disk Yokogawa CXU-X1 A1 (DND-22 experiment only). Data were analyzed with ImageJ software (NIH).

Western blotting. Cells were treated as indicated in Fig. 3/4, then washed twice with PBS and lysed with 2× Laemmli buffer. Cell extracts were heated for 5 min at 95 °C, sheared through a 26-gauge needle and quantified with a Nanodrop 2000 (Thermo Scientific). Protein lysates (~100 µg) were loaded on a 4–20% Mini-PROTEAN® TGX Stain-Free™ Gel (BioRad), resolved by SDS-PAGE electrophoresis and transferred onto a nitrocellulose membrane (Amersham). Proteins were probed with indicated antibodies: anti-β-actin (ab8226, Abcam), anti-H2AX (PA1-14198, Thermo Scientific), anti-γH2AX (#2577, Cell Signaling), anti-p21 (#2947, Cell Signaling), anti-p53 (#2524, Cell Signaling), anti-p-p53 (#9284, Cell Signaling), anti-p62 (610833, BD Transduction Laboratories™), anti-LC3

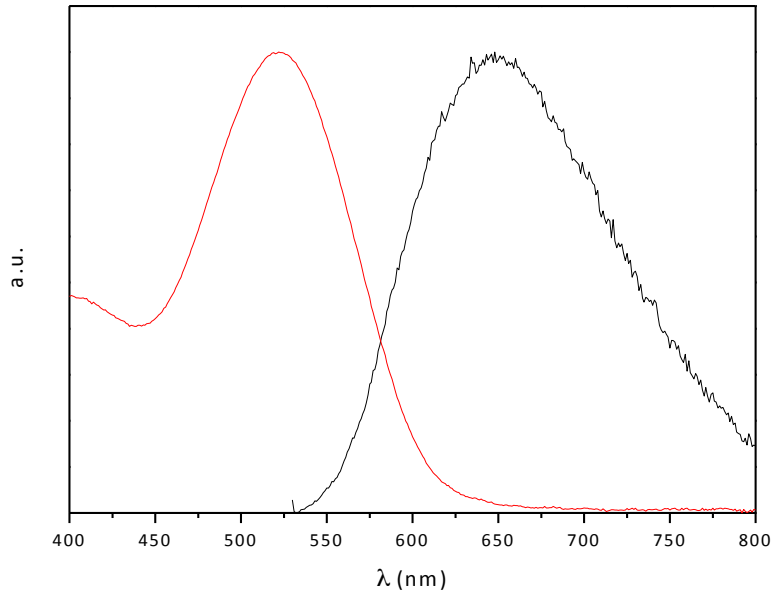
(#2775, Cell Signaling), anti-Bid (#2002, Cell Signaling), anti-Caspase 3 (#9665, Cell Signaling) and were diluted 1/1,000 in 5% BSA, 0.1% Tween-20/TBS. Secondary antibodies were anti-rabbit HRP (A120-108P, Bethyl), anti-mouse HRP (A90-116P) and anti-donkey HRP (A140-107P, Bethyl). Antigens were detected by ECL (Amersham). Imaging was performed using a ChemiDoc™ XRS+ System.



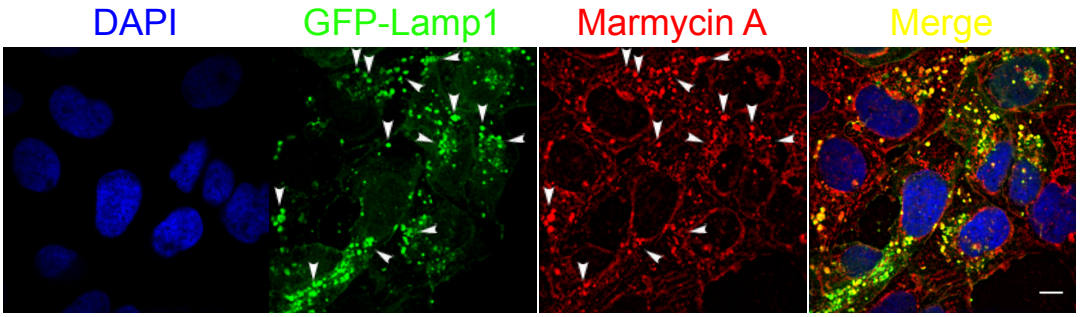
Supplementary Figure S1 | Percentage of viable A2780 cells after 72 h treatment with doxorubicin (**5**) or marmycin A (**1**). *n* = 3; error bars, s.d.



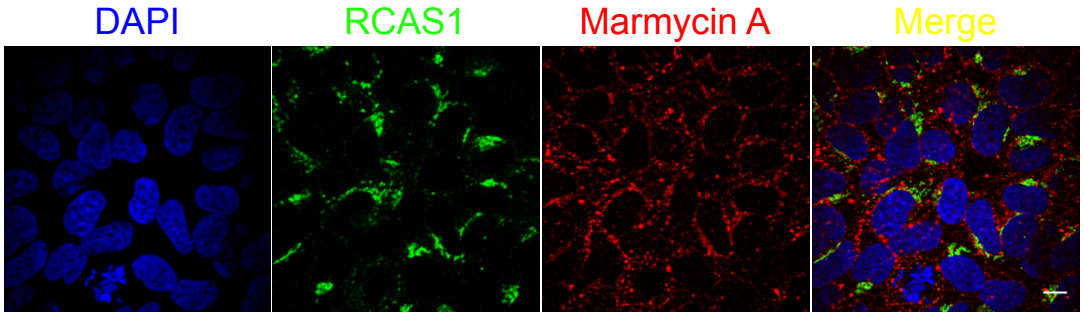
Supplementary Figure S2 | Fluorescence-activated cell sorting (FACS) analysis of A2780 cells either untreated or treated with 50 μM marmycin A (**1**). *x*- and *y*-axis, arbitrary units.



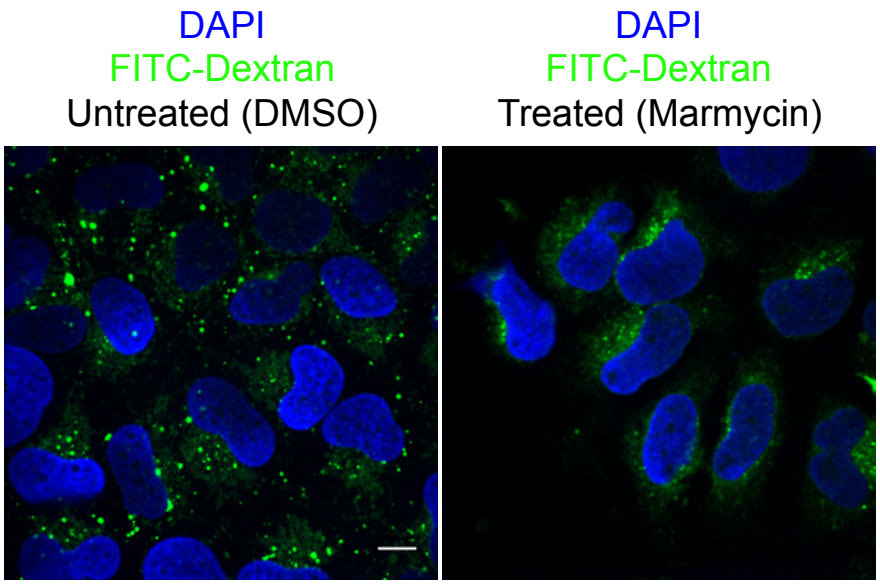
Supplementary Figure S3 | Fluorescence Ex/Em spectra of marmycin A (**1**) in methanol. a.u., arbitrary unit.



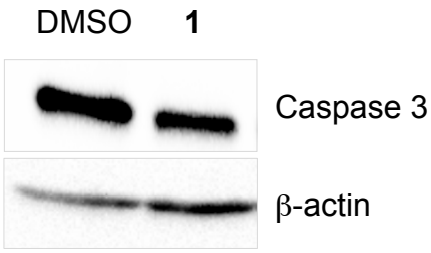
Supplementary Figure S4 | Fluorescence microscopy images of U2OS cells expressing GFP-Lamp1 (green) treated with **1** (red) showing the accumulation of **1** in lysosomes (yellow). White arrowheads indicate sites of colocalization. Scale bar, 10 μm.



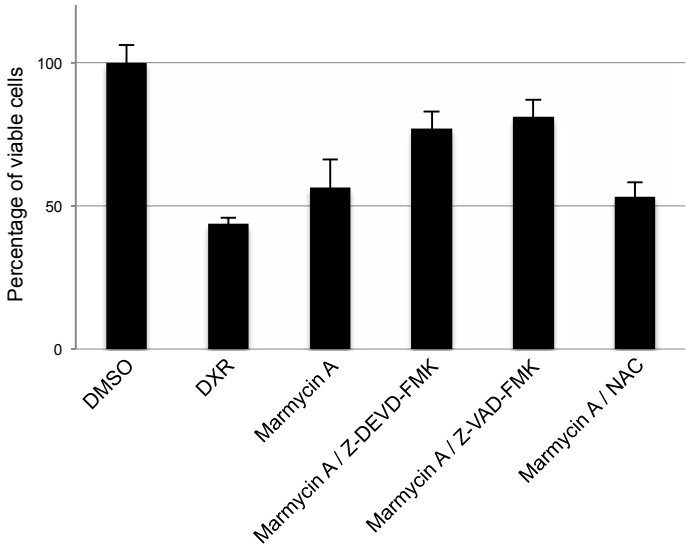
Supplementary Figure S5 | Fluorescence microscopy images of U2OS cells treated with **1** (red) showing no colocalization of **1** with the golgi apparatus protein RCAS1 (green). Scale bar, 10 μm.



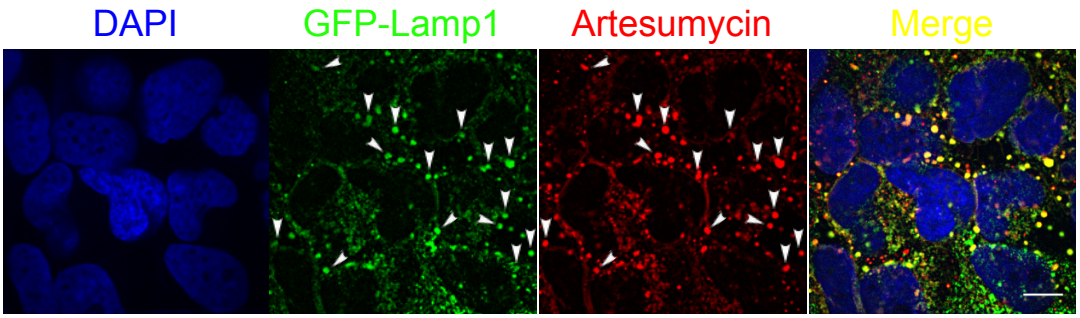
Supplementary Figure S6 | Fluorescence microscopy images of U2OS cells showing the subcellular distribution of the lysosomotropic agent FITC-Dextran (green). Left panel indicates a predominant lysosomal accumulation in untreated cells. Right panel indicates a release of FITC-Dextran from lysosome in cells treated with **1**. Scale bar, 10 μm.



Supplementary Figure S7 | Reduction of pro-caspase 3 upon treatment with 10 μM marmycin A (**1**) for 96 h.



Supplementary Figure S8 | Histogram showing the percentage of viable U2OS cells treated with DMSO, DXR (1 μM, 24h) and **1** (50 μM, 24h) in the presence of caspase 3 inhibitors or NAC. *n* = 4; error bars, s.d.



Supplementary Figure S9 | Fluorescence microscopy images of U2OS cells expressing GFP-Lamp1 (green) treated with **21** (red) showing the accumulation of **21** in lysosomes (yellow). White arrowheads indicate sites of colocalization. Scale bar, 10 μm.

3. References

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