Supporting Information:

Targeting Anthracycline-Resistant Tumor Cells with Synthetic Aloe-Emodin Glycosides

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[£] We dedicate this paper to the memory of our dear colleague, Professor Eliezer Flescher.

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Contents:

1. *General methods, cell strains, plasmids, materials, and instrumentation*

General Techniques: NMR spectra were recorded on Bruker instruments: Avance™ 400 (400 MHz for ^{1}H, 100.6 MHz for ^{13}C) or Avance™ 500 (500 MHz for ^{1}H, 125.7 MHz for ^{13}C). Chemical shifts are reported in unit parts per million (ppm). ^{1}H NMR spectra were calibrated as follows: CD_3OD (3.34 ppm). ^{13}C NMR spectra were calibrated as follows: CD_3OD (49.86 ppm). Multiplicities are reported by using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, ddd = double double doublet, dq = double quartet. Coupling constants (*J*) are given in Hertz. Unless otherwise mentioned, all reactions were conducted under argon atmosphere using anhydrous solvents. Reactions were monitored by electron spray ionization mass spectrometry (ESI-MS) and recorded on a Waters 3100 mass detector. High-resolution mass spectra were measured on a Waters Synapt instrument. AEGs were purified on an ECOM preparative HPLC system using a Phenomenex Luna axia 5 μm C-18 column (250 mm x 21.20 mm). Size exclusion chromatography was performed on an LH-20 column (70 cm x 1.4 cm). All reagents were used without further purification and were purchased from Sigma Aldrich, Alfa aeser, and Carbosynth. Aloe-emodin was purchased from Molekula. The cell lines used in this study, MOLT-4 (acute lymphoblastic leukemia), OVCAR3 and NAR (ovarian carcinomas) and MCF7 (breast adenocarcinoma), were purchased from ATCC (Manassas, VA). Cell culture supplies were purchased from Biological Industries, Beit-Haemek, Israel. PBR322 plasmid was purchased from Fermentas. DNA gels were run on a Bio-Rad Laboratories Ltd. instrument. $DiIc_{18}(5)$-DS plasma membrane stain was purchased from Invitrogen. Imaging was performed using an Andor Revolution Imaging System equipped with a Yokogawa CSU-X1 Spinning Disk Unit and an iXon 897 back-illuminated EMCCD camera mounted on a custom made Olympus IX-Upright microscope.

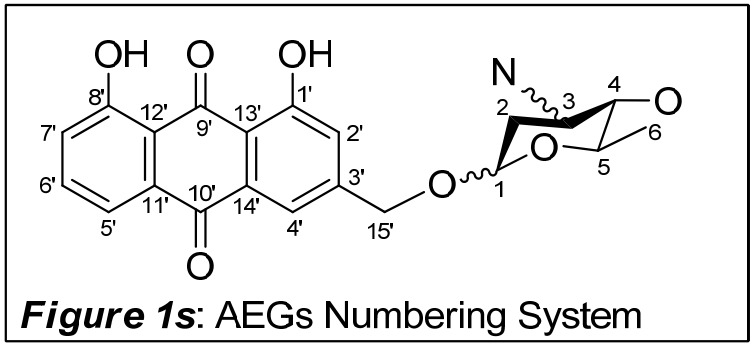
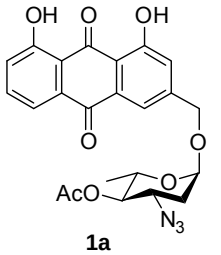


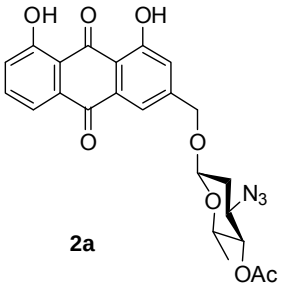
Figure 1s: AEGs Numbering System

2. *Synthetic procedures*

The numbering system used to describe the AE analogs is shown in Figure 1s.



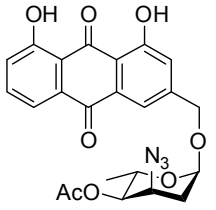
1a



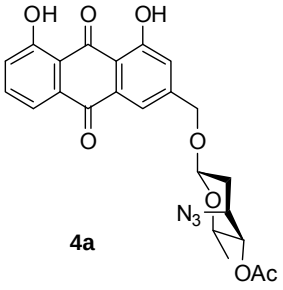
2a

AEGs 1a and 2a: Acosamine glycosyl donor **D-1** (295.0 mg, 1.15 mmol) and AE (283.4 mg, 1.05 mmol) in dry THF (6.0 ml) were added to flame-dried molecular sieves (4 Å, 400 mg) and stirred under argon atmosphere at ambient temperature for 20 min. The reaction mixture was then cooled to 0°C and trimethylsilyl trifluoromethanesulfonate (60 μL, 0.33 mmol) was added. Reaction progress was monitored by TLC (70% petroleum ether, 30% ethyl acetate) and indicated the formation of an anomeric mixture of products (α-anomer **1a** R_f=0.71, (β-anomer **2a** R_f=0.57). Upon completion (40 h at 0°C), the reaction was quenched by trimethylamine (60 μL) and the crude was filtered through a small plug of celite. The products were isolated by reverse-phase HPLC using a Phenomenex Luna axia 5 μm C-18 (250 mm x 21.20 mm) column at a flow rate of 20.0 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 80% B for 2 min followed by 80-100% B over 20 min and product elution was monitored at 256 nm by a UV detector. The product retention times were 9.4 minutes for the α-anomer **1a** and 8.8 minutes for the β-anomer **2a**. Fractions containing the pure product were concentrated under reduced pressure to yield α-anomer 1a (162.4 mg, 0.35 mmol), β-anomer 2a (66.3 mg, 0.14 mmol). The total isolated yield of the reaction was 43%. ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **1a** δ: 12.01 (s, 1H, O*H*), 11.99 (s, 1H, O*H*) 7.77 (d, *J*=7.5 Hz, 1H, H-5'), 7.67 (s, 1H, H-4), 7.62 (t, *J*=7.8 Hz, 1H, H-6), 7.22 (m, 2H, H-2, H-7'), 4.93 (bd, *J*=3.2 Hz, 1H, H-1), 4.68 (d, *J*=13.6 Hz, 1H, H-15'), 4.64 (dd, J_1=J_2=9.8 Hz, 1H, H-4), 4.48 (d, *J*=13.7 Hz, 1H, H-15'), 3.87 (ddd, J_1=4.9, J_2=9.9 J_3=12.3 Hz, 1H, H-3), 3.75 (dq, J_1=6.3, J_2=9.6 Hz,1H, H-5), 2.22 (bdd, J_1=4.9, J_2=13.3 Hz, 1H, H-2eq), 2.06 (s, 3H, CO*CH$_3$*), 1.74 (dd, J_1=3.5, J_2=12.9 Hz, 1H, H-2ax), 1.11 (d, *J*=6.3 Hz, 3H, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$) for AEG 1a δ: 192.1 (C-9'), 181.1 (C-10'), 169.6 (*CO*CH$_3$), 162.3, 162.1, 147.6 (C-3'), 136.8 (C-6'), 133.2, 133.0, 124.3, 121.7, 119.6, 117.9, 115.2, 114.5, 95.7 (C-1), 74.8, 67.3, 65.9, 57.0, 34.5(C-2), 20.6 (CO*CH$_3$*), 16.9 (C-6). Positive HRESIMS, *m/z* calcd 490.1226 for $C_{23}H_{21}N_3O_8Na$, found 490.1231 $[M+Na]^+$.

^{1}H NMR (500 MHz, $CDCl_3$) for AEG **2a** δ: 11.94 (s, 1H, O*H*), 11.93 (s, 1H, O*H*) 7.72 (d, *J*=7.4 Hz, 1H, H-5'), 7.64 (s, 1H, H-4'), 7.59 (t, *J*=7.9 Hz, 1H, H-6'), 7.20 (m, 2H, H-2', H-7'), 4.88 (d, *J*=13.7 Hz, 1H, H-15'), 4.63 (dd, J_1= J_2=9.6 Hz, 1H, H-4), 4.58 (d, *J*=13.7 Hz, 1H, H-15'), 4.56 (dd, J_1-=1.8, J_2=9.7 Hz, 1H, H-1), 3.48 (ddd, J_1=5.0, J_2=9.8, J_3=12.8 Hz, 1H, H-3), 3.40 (dq, J_1=6.2, J_2=9.5 Hz, 1H, H-5), 2.27 (ddd, J_1=1.4, J_2=4.8, J_3=12.9 Hz, 1H, H-2eq), 2.06 (s, 3H, OCH_3), 1.72 (ddd, J_1= J_2=9.7, J_3=12.7 Hz, 1H, H-2ax), 1.18 (d, J_1=6.2 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$) for AEG 2a δ: 193.3 (C-9'), 182.3 (C-10'), 170.7 (*CO*CH_3), 163.5, 163.2, 148.9 (C-3'), 137.9 (C-6'), 134.3, 134.2, 125.4, 122.9, 120.8, 119.1, 116.5, 115.7, 99.5 (C-1), 75.6, 71.7, 69.9, 60.4, 36.8 (C-2), 21.6 (CO*CH$_3$*), 18.2 (C-6). Positive HRESIMS, *m/z* calcd 490.1226 for $C_{23}H_{21}N_3O_8Na$, found 490.1224 $[M+Na]^+$.



3a

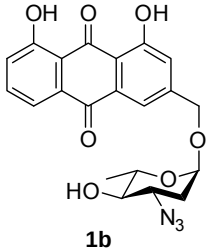


4a

AEGs 3a and 4a: Ristosamine glycosyl donor **D-2** (295.0 mg, 1.15 mmol) and AE (283.4 mg, 1.05 mmol) in dry THF (6.0 ml) were added to flame-dried molecular sieves (4 Å, 400 mg) and stirred under argon atmosphere at ambient temperature for 20 min. The reaction mixture was then cooled to 0°C and trimethylsilyl trifluoromethanesulfonate (60 μL, 0.33 mmol) was added. Reaction progress was monitored by TLC (70% petroleum ether, 30% ethyl acetate), which indicated the formation of an anomeric mixture of products (α–anomer **3a** R_f=0.51, β–anomer **4a** R_f=0.69). Upon completion (18 h at 0°C), the reaction was quenched by trimethylamine (60 μL) and the crude was filtered through a small plug of celtie. The products were isolated by reverse-phase HPLC using a Phenomenex Luna axia 5 μm C-18 (250 mm x 21.20 mm) column at a flow rate of 20.0 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 80% B for 2 min followed by 80-100% B over 20 min and product elution was monitored at 256 nm by a UV detector. The product retention times were 8.1 minutes for the α–anomer 3a and 9.4 minutes for the β-anomer **4a**. Fractions containing the pure product were concentrated under reduced pressure to yield the pure α-anomer **3a** (113.4 mg, 0.24 mmol), the pure β–anomer **4a** (182.7 mg, 0.39 mmol). The total isolated yield of the reaction was 69% . ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **3a** δ: 12.02 (s, 1H, OH), 12.01 (s, 1H, OH), 7.78 (d, *J*=7.4

Hz, 1H, H-5'), 7.74 (s, 1H, H-4'), 7.62 (t, *J*=8.0 Hz, 1H, H-6'), 7.32 (s, 1H, H-2'), 7.24 (d, *J*=8.4 Hz, 1H, H-7'), 4.85 (d, *J*=4.0 Hz, 1H, H-1), 4.77 (d, *J*=14.0 Hz, 1H, H-15'), 4.62 (dd, J_1=3.5, J_2=9.6 Hz, 1H, H-4), 4.53 (d, *J*=14.0 Hz, 1H, H-15'), 4.17 (dq, J_1=6.3, J_2=9.5, 1H, H-5), 4.12 (ddd, J_1= J_2= J_3=3.5 Hz, 1H, H-3), 2.20 (bdd, J_1=3.0, J_2=14.9 Hz, 1H, H-2eq), 2.07 (s, 3H, OCH3), 2.05 (ddd, J_1= J_2=4.2, J_3=14.7 Hz, 1H, H-2ax), 1.11 (d, *J*=6.3 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$) for AEG **3a** δ: 193.4 (C-9'), 182.4 (C-10'), 170.9 (*CO*CH_3), 163.6, 163.3, 149.5 (C-3'), 137.9 (C-6'), 134.4 (C-11', C-14'), 125.4, 122.9, 120.8, 119.2, 116.6, 115.7, 95.9 (C-1), 74.5, 68.7, 63.0, 56.3, 30.4 (C-2), 21.5 (CO*CH_3*), 18.0 (C-6). Positive HRESIMS, *m/z* calcd 490.1226 for $C_{23}H_{21}N_3O_8Na$, found 490.1226 $[M+Na]^+$.^{1}H NMR (500 MHz, $CDCl_3$)

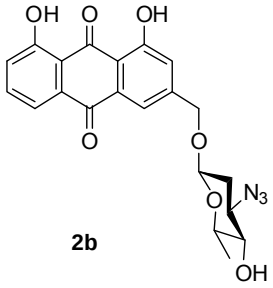
For AEG **4a** δ: 12.01 (s, 1H, O*H*), 11.99 (s, 1H, O*H*) 7.77 (d, *J*=7.5 Hz, 1H, H-5'), 7.70 (s, 1H, H-4'), 7.63 (t, *J*=8.0 Hz, 1H, H-6') 7.23 (d, *J*=7.9 Hz, 1H, H-7'), 7.19 (s, 1H, H-2'), 4.89 (d, *J*=13.6 Hz, 1H, H-15'), 4.77 (dd, J_1=2.0, J_2=8.9 Hz, 1H, H-1), 4.64 (dd, J_1=3.4, J_2=9.2 Hz, 1H, H-4), 4.58 (d, *J*=13.6 Hz, 1H, H-15'), 4.15 (ddd, J_1= J_2= J_3=3.5 Hz, 1H, H-3), 3.93 (dq, J_1=6.3, J_2=9.2 Hz, 1H, H-5), 2.08 (ddd, J_1=2.2, J_2=4.0, J_3=12.0 Hz, 1H, H-2eq), 2.07 (s, 3H, OC*H_3*), 1.87 (ddd, J_1=3.4, J_2=9.0, J_3=12.4 Hz, 1H, H-2ax), 1.19 (d, *J*=6.4 Hz, 3H, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$) for AEG **4a** δ: 192.2(C-9'), 181.2 (C-10'), 169.6 (*CO*CH_3), 162.3, 162.0, 148.0 (C-3'), 136.7 (C-6'), 133.1 (C-11', C-14'), 124.2, 121.8, 119.6, 118.0, 115.4, 114.5, 96.7 (C-1), 73.8, 68.7, 67.8, 57.1, 34.8 (C-2), 20.2 (CO*CH_3*), 17.3 (C-6). Positive HRESIMS, *m/z* calcd 490.1226 for $C_{23}H_{21}N_3O_8Na$, found 490.1225 $[M+Na]^+$.



1b

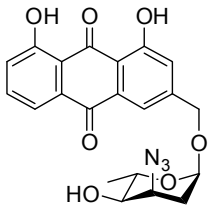
AEG 1b: AEG **1a** (160.0 mg, 0.34 mmol) in MeOH:DCM/9:1 (5 mL) was added to K_2CO_3 (50.0 mg, 0.36 mmol) and stirred at ambient temperature. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 466.5) and formation of AEG **1b** ($[M-H]^-$, *m/z* 424.5). After 20 h, acetic acid was added dropwise until the crimson red solution turned yellow. The volume of the crude mixture was reduced under vacuum to 1 mL, and products were separated by size-exclusion chromatography (Sephadex LH-20 loaded on a 700-mm length, 11.5-mm diameter column). The column was loaded and eluted with MeOH/DCM (1:1). Fractions containing the pure product were concentrated to yield AEG **1b** as yellow powder (126.6 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **1b** δ: 12.01 (s, 1H, OH), 12.00 (s, 1H, OH) 7.77(d, *J*=7.3 Hz, 1H, H-5'), 7.70 (s, 1H, H-4'), 7.62 (t, *J*=7.8 Hz, 1H, H-6'),

7.25-7.22 (m, 2H, H-2', H-7'), 4.92 (bd, J=3.2 Hz, 1H, H-1), 4.70 (d, J=13.7 Hz, 1H, H-15'), 4.49 (d, J=13.7 Hz, 1H, H-15'), 3.77 (ddd, J_1=4.9, J_2=9.5, J_3=12.3 Hz, 1H, H-3), 3.66 (dq, J=6.2, J=9.2 Hz, 1H, H-5), 3.12 (dd, J_1=J_2=9.4 Hz, 1H, H-4), 2.22 (bdd, J_1=4.9, J_2=13.2 Hz, 1H, H-2eq), 1.72 (ddd, J_1=3.6, J_2=J_3=12.9 Hz, 1H, H-2ax), 1.25 (d, J=6.3 Hz, 3H, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$) for AEG **1b** δ: 192.1 (C-9'), 181.1 (C-10'), 162.3, 162.1, 147.8 (C-3'), 136.7 (C-6'), 133.2, 133.1, 124.2, 121.7, 119.6, 118.0, 115.2, 114.5, 95.8 (C-1), 75.3, 67.7, 67.2, 59.7, 34.3 (C-2), 17.2 (C-6). Positive HRESIMS, *m/z* calcd 448.1121 for $C_{21}H_{19}N_3O_7Na$, found 448.1119 $[M+Na]^+$.



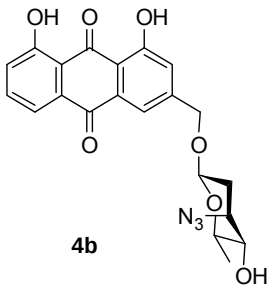
2b

AEG 2b: AEG **2a** (58.3 mg, 0.12 mmol) in MeOH:DCM/9:1 (5 mL) was added to K_2CO_3 (50.0 mg, 0.36 mmol) and stirred at ambient temperature. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 466.5) and formation of AEG **2b** ($[M-H]^-$, *m/z* 424.5). After 20 h, acetic acid was added dropwise until the crimson red solution turned yellow. The volume of the crude mixture was reduced under vacuum to 1 mL, and products were separated by size-exclusion chromatography (Sephadex LH-20 loaded on a 700-mm length, 11.5-mm diameter column). The column was loaded and eluted with MeOH/DCM (1:1). Fractions containing the pure product were concentrated to yield AEG **2b** as yellow powder (47.8 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **2b** δ: 12.01 (s, 1H, OH), 11.97 (s, 1H, OH), 7.77 (d, J=7.4 Hz, 1H, H-5'), 7.71 (s, 1H, H-4'), 7.62 (t, J=7.9 Hz, 1H, H-6'), 7.20 (m, 2H, H-2', H-7'), 4.91 (d, J=13.7 Hz, 1H, H-15'), 4.61 (d, J=13.7 Hz, 1H, H-15'), 4.57 (dd, J_1=1.6, J_2=9.6 Hz, 1H, H-1), 3.37 (ddd, J_1=4.9, J_2=9.4, J_3=12.4 Hz, 1H, H-3), 3.30 (dq, J_1=6.1, J_2=9.0 Hz, 1H, H-5), 3.12 (ddd, J_1=3.5, J_2= J_3=9.1 Hz, 1H, H-4), 2.26 (ddd, J_1=1.7, J_2=4.9, J_3=12.8 Hz, 1H, H-2eq), 2.16 (d, J_1 =3.7 Hz, 1H, OH in C-4), 1.70 (ddd, J_1= 9.7, J_2=J_3=12.6, Hz, 1H, H-2ax), 1.31 (d, J=6.1 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$) for AEG **2b** δ: 207.7 (C-9'), 182.4 (C-10'), 163.6, 163.3, 149.1 (C-3'), 137.9 (C-6'), 134.4, 134.3, 125.5, 123.0, 120.8, 119.3, 116.6, 115.8, 99.5 (C-1), 76.2, 73.4, 69.9, 63.2, 31.6 (C-2), 18.5 (C-6). Positive HRESIMS, *m/z* calcd 448.1121 for $C_{21}H_{19}N_3O_7Na$, found 448.1123 $[M+Na]^+$.



3b

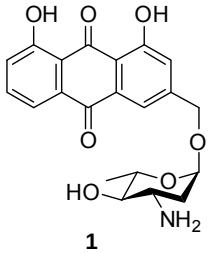
AEG 3b: AEG **3a** (113.4 mg, 0.24 mmol) in MeOH:DCM/9:1 (5 mL) was added to K_2CO_3 (50.0 mg, 0.36 mmol) and stirred at ambient temperature. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 466.5) and formation of AEG **3b** ($[M-H]^-$, *m/z* 424.5). After 20 h, acetic acid was added dropwise until the crimson red solution turned yellow. The volume of the crude mixture was reduced under vacuum to 1 mL, and products were separated by size-exclusion chromatography (Sephadex LH-20 loaded on a 700-mm length, 11.5-mm diameter column). The column was loaded and eluted with MeOH/DCM (1:1). Fractions containing the pure product were concentrated to yield AEG **3b** as yellow powder (98.0 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **3b** δ: 12.02 (s, 1H, OH), 12.01 (s, 1H, OH) 7.77 (d, *J*=7.4 Hz, 1H, H-5'), 7.75 (s, 1H, H-4'), 7.62 (t, *J*=8.0 Hz, 1H, H-6'), 7.30 (s, 1H, H-2'), 7.25 (d, *J*=8.6 Hz, 1H, H-7'), 4.86 (d, *J*=4.0 Hz, 1H, H-1), 4.77 (d, *J*=14.0 Hz, 1H, H-15'), 4.51 (d, *J*=14.0 Hz, 1H, H-15'), 4.07 (ddd, J_1= J_2= J_3=3.6 Hz, 1H, H-3), 3.84 (dq, J_1=6.3, J_2=9.3 Hz, 1H, H-5), 3.29 (dd, J_1=3.5, J_2=9.2 Hz, 1H, H-4), 2.31 (bdd, J_1=2.3, J_2=15.1 Hz, 1H, H-2eq), 2.05 (ddd, J_1= J_2=4.2, J_3=15.2 Hz, 1H, H-2ax), 1.20 (d, *J*=6.3 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$) for AEG **3b** δ: 193.4 (C-9'), 182.4 (C-10'), 163.6, 163.2, 149.7 (C-3'), 137.9 (C-6'), 134.3 (C-11', C-14'), 125.4, 122.7, 120.8, 119.0, 116.6, 115.6, 95.7 (C-1), 72.6, 68.6, 65.6, 58.7, 32.8 (C-2), 18.2 (C-6). Positive HRESIMS, *m/z* calcd 448.1121 for $C_{21}H_{19}N_3O_7Na$, found 448.1118 $[M+Na]^+$.



4b

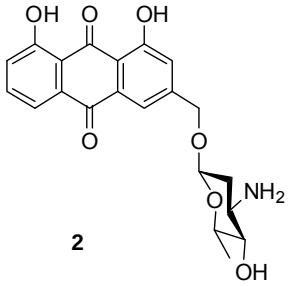
AEG 4b: AEG **4a** (182.7 mg, 0.39 mmol) in MeOH:DCM/9:1 (5 mL) was added to K_2CO_3 (50.0 mg, 0.36 mmol) and stirred at ambient temperature. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 466.5) and formation of AEG **4b** ($[M-H]^-$, *m/z* 424.5). After 20 h, acetic acid was added dropwise until the crimson red solution turned yellow. The volume of the crude mixture was reduced under vacuum to 1 mL, and products were separated by size-exclusion chromatography (Sephadex LH-20 loaded on a 700-mm length, 11.5-mm diameter column). The column was loaded and eluted with MeOH/DCM (1:1). Fractions containing the pure product were concentrated to yield AEG **4b** as yellow powder (130.0 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) for AEG **4b** δ: 12.00 (s, 1H, O*H*), 11.98 (s, 1H, O*H*), 7.75(d, *J*=7.4 Hz, 1H,

H-5'), 7.69 (s, 1H, H-4'), 7.61 (t, J=7.9 Hz, 1H, H-6'), 7.25-7.22 (m, 2H, H-2', H-7'), 4.90 (d, J=13.7 Hz, 1H, H-15'), 4.78 (dd, J_1=2.0, J_2=9.0 Hz, 1H, H-1), 4.57 (d, J=13.7 Hz, 1H, H-15'), 4.06 (ddd, J_1= J_2= J_3=3.5 Hz, 1H, H-3), 3.63 (dq, J_1=6.3, J_2=8.9 Hz, 1H, H-5), 3.39 (dd, J_1=3.0, J_2=8.6 Hz, 1H, H-4), 2.19 (ddd, J_1=2.2, J_2=3.7, J_3=14.0 Hz, 1H, H-2eq), 1.87 (ddd, J_1=3.4, J_2=9.1, J_3 =13.9 Hz, 1H, H-2ax), 1.27 (d, J=6.3 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$) for AEG **4b** δ: 192.2 (C-9'), 181.2 (C-10'), 162.3, 162.0, 148.0 (C-3'), 136.7 (C-6'), 133.1 (C-11', C-14'), 124.2, 121.8, 119.6, 118.0, 115.3, 114.4, 96.6 (C-1), 72.0, 70.2, 68.7, 60.3 (C-5 or C-15' or C-4 or C-3), 34.7 (C-2), 17.4 (C-6). Positive HRESIMS, *m/z* calcd 448.1121 for $C_{21}H_{19}N_3O_7Na$, found 448.1122 $[M+Na]^+$.



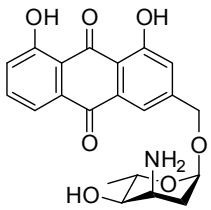
1

AEG 1: AEG **1b** (20.6 mg, 48 μmol) dissolved in MeOH:DCM/5:1 (2.5 mL) was added to palladium on carbon (10.0 mg), trifluoroacetic acid (10 μL) and stirred at ambient temperature under a hydrogen balloon. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 424.5) and formation of AEG **1** ($[M-H]^-$, *m/z* 398.5). After 15 min, the mixture was filtered (PHENEX PTFE Membrane, 0.2 μm, 15 mm Syringe Filters) and purified by HPLC using a Phenomenex Luna C18 HPLC column at a flow rate of 20 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 50% B for 2 min followed by 50-100% B over 15 min. Product elution was monitored at 256 nm. The product was detected after 3.9 min. Fractions containing the pure product were concentrated under vacuum, dissolved in H_2O, and freeze-dried to yield AEG **1** as a yellow powder (10.7 mg, 75%).^{1}H NMR (500 MHz, MeOH-d_4) for AEG **1** δ: 7.66-7.75 (m, 3H, H-4', H-5', H-6'), 7.27 (d, J=8.1 Hz, 1H, H-7'), 7.24 (s, 1H, H-2'), 5.00 (bd, J=3.0 Hz, 1H, H-1), 4.72 (d, J=13.7 Hz, 1H, H-15'), 4.57 (d, J=13.7 Hz, 1H, H-15''), 3.64 (dq, J_1=6.2, J_2=9.1 Hz, 1H, H-5), 3.40 (ddd, J_1=4.5, J_2=10.1, J_3=12.7 Hz, 1H, H-3), 3.09 (dd, J_1= J_2=9.6 Hz, 1H, H-4), 2.24 (bdd, J_1=4.5, J_2=12.9 Hz, 1H, H-2eq), 1.82 (ddd, J_1=3.5, J_2=J_3=12.8 Hz, 1H, H-2ax), 1.21 (d, J=6.2 Hz, 3H, H-6). ^{13}C NMR (125.7 MHz, MeOH-d_4) for AEG **1** δ: 192.0 (C-9'), 181.0 (C-10'), 161.9, 161.7, 148.1 (C-3'), 136.5 (C-6'), 133.2, 133.1, 123.7, 121.2, 118.8, 117.3, 115.2, 114.4, 95.1(C-1), 72.4, 68.2, 67.0, 49.5, 34.3 (C-2), 17.2 (C-6). Positive HRESIMS, *m/z* calcd 400.1396 for $C_{21}H_{22}NO_7$, found 400.1396 $[M+H]^+$.



2

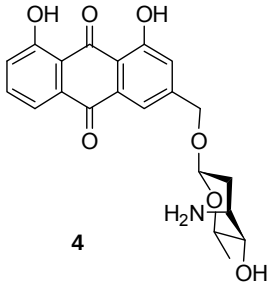
AEG 2: AEG **2b** (17.0 mg, 40 μmol) dissolved in MeOH:DCM/5:1 (2 mL) was added to palladium on carbon (10.0 mg), trifluoroacetic acid (10.0 μL) and stirred at ambient temperature under a hydrogen balloon. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ([M-H]$^-$, *m/z* 424.5) and formation of AEG **2** ([M-H]$^-$, *m/z* 398.5). After 15 min, the mixture was filtered (PHENEX PTFE Membrane, 0.2 µm, 15 mm Syringe Filters) and purified by HPLC using a Phenomenex Luna C18 HPLC column at a flow rate of 20 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 50% B for 2 min followed by 50-100% B over 15 min. Product elution was monitored at 256 nm. The product was detected after 4.0 min. Fractions containing the pure product were concentrated under vacuum, dissolved in H_2O, and freeze-dried to yield AEG **2** as a yellow powder (6.1 mg, 80%).^{1}H NMR (500 MHz, MeOH-d_4) for AEG **2** δ: 7.65-7.74 (m, 3H, H-4', H-5', H-6'), 7.26 (d, *J*=8.0 Hz, 1H, H-7'), 7.21 (s, 1H, H-2'), 4.88 (d, *J*=13.7 Hz, 1H, H-15), 4.72 (dd, J_1=1.9, J_2=9.4 Hz, 1H, H-1), 4.66 (d, *J*=13.7 Hz, 1H, H-15'), 3.33 (dq, J_1=6.2, J_2=8.8 Hz, 1H, H-5), 3.14 (ddd, J_1=4.6, J_2=9.9, J_3=12.3 Hz, 1H, H-3), 3.07 (dd, J_1=J_2=9.3 Hz, 1H, H-4), 2.27 (ddd, J_1=1.7, J_2=4.4, J_3=12.1 Hz, 1H, H-2eq), 1.67 (ddd, J_1=9.5, J_2= J_3=12.3 Hz, 1H, H-2ax), 1.27 (d, *J*=6.2 Hz, 3H, H-6). ^{13}C NMR (125.7 MHz, MeOH-d_4) for AEG **2** δ: 192.0 (C-9'), 180.9 (C-10'), 161.8, 161.7, 148.2 (C-3'), 136.5 (C-6'), 133.1, 133.0, 123.7, 121.1, 118.8, 117.3, 115.2, 114.3, 98.1 (C-1), 72.6, 72.2, 68.5, 51.6, 34.5 (C-2), 16.0 (C-6). Positive HRESIMS, *m/z* calcd 400.1396 for $C_{21}H_{22}NO_7$, found 400.1393 $[M+H]^+$.



3

AEG 3: AEG **3b** (20.0 mg, 47 μmol) dissolved in MeOH:DCM/5:1 (2 mL) was added to palladium on carbon (10.0 mg), trifluoroacetic acid (10 μL) and stirred at ambient temperature under a hydrogen balloon. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ([M-H]$^-$, *m/z* 424.5) and formation of AEG **3** ([M-H]$^-$, *m/z* 398.5). After 15 min, the mixture was filtered (PHENEX PTFE Membrane, 0.2 µm, 15 mm Syringe Filters) and purified by HPLC using a Phenomenex Luna C18 HPLC column at a flow rate of 20 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 50% B for 2 min followed by 50-100% B over 15 min. Product elution was monitored at 256 nm. The product was detected after 4.0 min. Fractions containing the pure product were concentrated

under vacuum, dissolved in H_2O, and freeze-dried to yield AEG **3** as a yellow powder (17.4 mg, 93%). ^{1}H NMR (500 MHz, MeOH-d_4) for AEG **3** δ: 7.65-7.73 (m, 3H, H-4', H-5', H-6'), 7.23-7.29 (m, 2H, H-2',H-7'), 4.91 (d, *J*=2.4 Hz, 1H, H-1), 4.76 (d, *J*=14.1 Hz, 1H, H-15'), 4.60 (d, *J*=14.1 Hz, 1H, H-15'), 3.82 (dq, J_1=6.1, J_2=9.6 Hz, 1H, H-5), 3.54 (ddd, J_1= J_2= J_3=3.6 Hz, 1H, H-3), 3.46 (dd, J_1=4.4, J_2=9.7 Hz, 1H, H-4), 2.16 (bd, *J*=15.3 Hz, 1H, H-2eq), 2.10 (ddd, J_1= J_2=3.8, J_3=15.2 Hz, 1H, H-2ax), 1.24 (d, *J*=6.1 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, MeOH-d_4) for AEG **3** δ: 193.3 (C-9'), 182.3 (C-10'), 163.1, 163.0, 149.0 (C-3'), 137.8 (C-6'), 134.4, 134.3, 125.0, 122.8, 120.1, 118.7, 116.4, 115.7, 96.3 (C-1), 68.9, 68.5, 64.7, 49.8, 31.9 (C-2), 17.5 (C-6). Positive HRESIMS, *m/z* calcd 400.1396 for $C_{21}H_{22}NO_7$, found 400.1394 $[M+H]^+$.



4

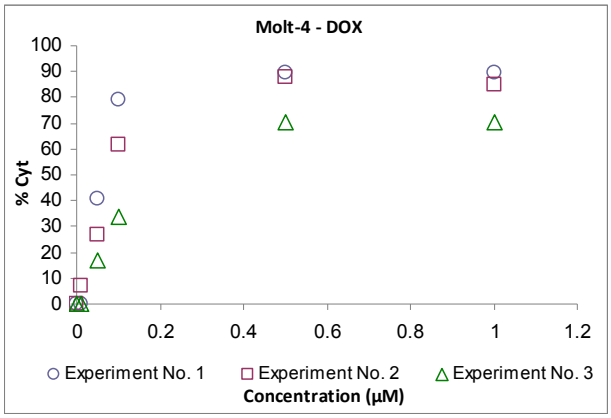
AEG 4: AEG **4b** (18.0 mg, 42 μmol) dissolved in MeOH:DCM/5:1 (2mL) was added to palladium on carbon (10.0 mg), trifluoroacetic acid (10 μL) and stirred at ambient temperature under a hydrogen balloon. Monitoring of the reaction by ESI-MS indicated the disappearance of the starting material ($[M-H]^-$, *m/z* 424.5) and formation of AEG **4** ($[M-H]^-$, *m/z* 398.5). After 15 min, the mixture was filtered (PHENEX PTFE Membrane, 0.2 μm, 15 mm Syringe Filters) and purified by HPLC using a Phenomenex Luna C18 HPLC column at a flow rate of 20 mL/min. HPLC solvent A was H_2O (0.1% TFA) and B was ACN (0.1% TFA). The elution gradient was 50% B for 2 min followed by 50-100% B over 15 min. Product elution was monitored at 256 nm. The product was detected after 4.0 min. Fractions containing the pure product were concentrated under vacuum, dissolved in H_2O, and freeze-dried to yield AEG **4** as a yellow powder (11.2 mg, 74%). ^{1}H NMR (500 MHz, MeOH-d_4) for AEG **4** δ: 7.61-7.68 (m, 3H, H-4', H-5', H-6'), 7.23 (dd, J_1=2.9, J_2=6.6 Hz, 1H, H-7'), 7.17 (s, 1H, H-2'), 4.93 (dd, J_1=2.9, J_2=6.0 Hz, 1H, H-1), 4.84 (d, *J*=13.9 Hz, 1H, H-15'), 4.59 (d, *J*=13.9, 1H, H-15''Hz), 3.79 (dq, J_1=J_2=6.5, Hz, 1H, H-5), 3.72 (ddd, J_1= J_2=4.3, J_3=7.4 Hz, 1H, H-3), 3.59 (dd, J_1=4.1, J_2=6.0 Hz, 1H, H-4), 2.19 (ddd, J_1=2.9, J_2=7.1, J_3=13.9 Hz, 1H, H-2eq), 1.99 (ddd, J_1 = J_2=4.9, J_3=13.9 Hz, 1H, H-2ax), 1.31 (d, *J*=6.6 Hz, 3H, H-6). ^{13}C NMR (100.6 MHz, MeOH-d_4) for AEG **4** δ: 193.2(C-9'), 182.0 (C-10'), 163.0, 162.9, 149.5 (C-3'), 137.7 (C-6'), 134.3, 134.2, 124.9, 122.3, 120.0, 118.4, 116.4, 115.5, 97.2 (C-1), 72.8, 69.0, 68.4, 49.1, 31.7 (C-2), 18.6 (C-6). Positive HRESIMS, *m/z* calcd 400.1396 for $C_{21}H_{22}NO_7$, found 400.1399 $[M+H]^+$.

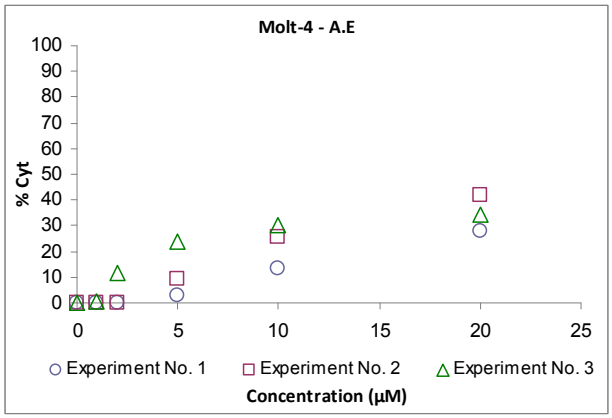
3. *IC_{50} protocol*

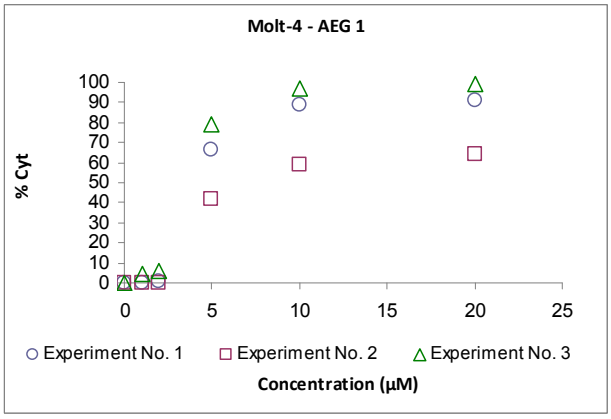
Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 1 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Cells were maintained in a humidified chamber of 95% air 5% CO_2 at 37°C. Cells (5 x 10^3/well) were plated into 96-well microtiter plates and were allowed to adhere (except for MOLT-4 cells) before treatment. After 24 hours, 5 μL of AEG, AE, or DOX solutions were added at various concentrations. The cells were incubated for 24 h. Cell viability was determined using an XTT kit (Biological Industries) as per the manufacturer's instructions. Optical density (OD) was read at 490 nm with a VERSAmax microplate ELISA reader (Molecular Devices). The IC_{50} was the concentration at which the OD value was 50% of the OD value of the untreated cells. Each concentration was evaluated in triplicate and each experiment was performed three times.

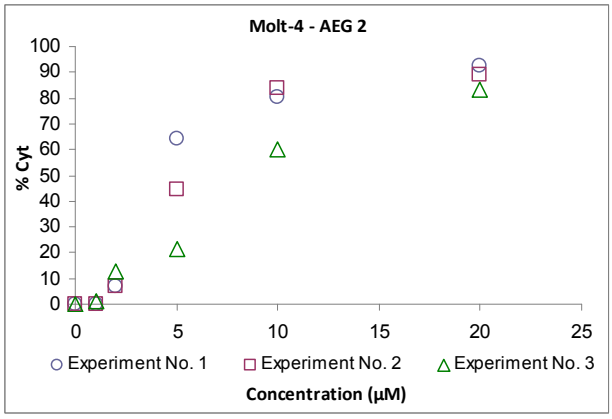
4. *IC_{50} curves*

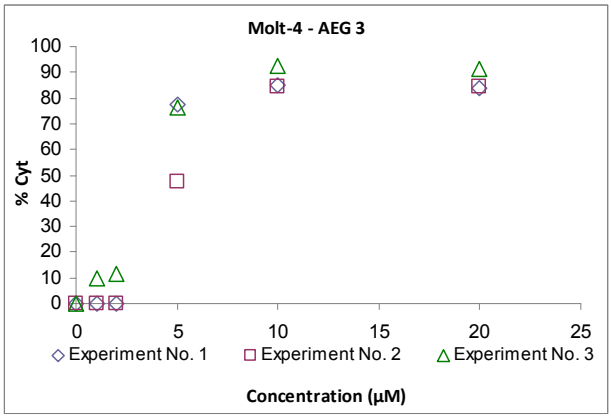
MOLT-4 IC_{50} Experiments:

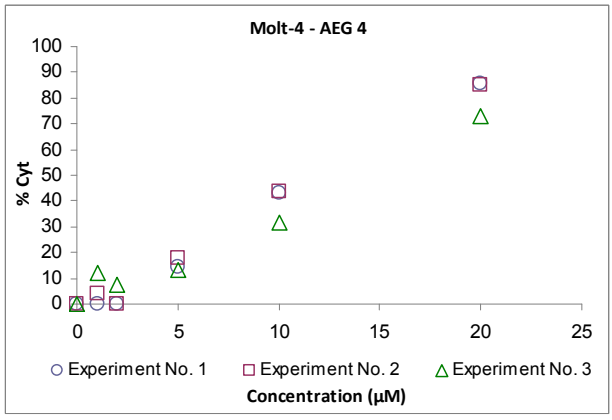




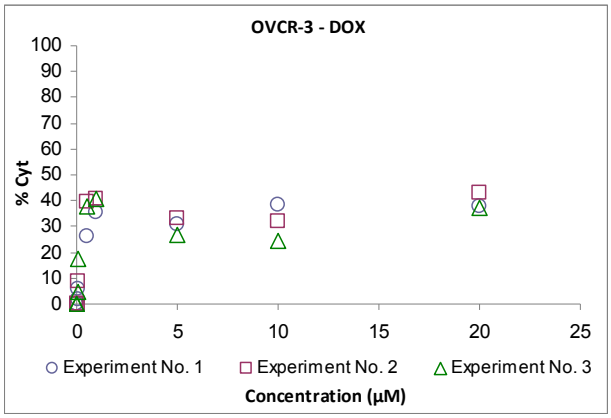


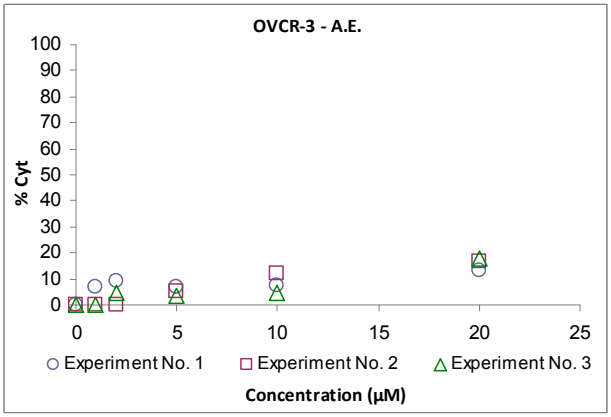


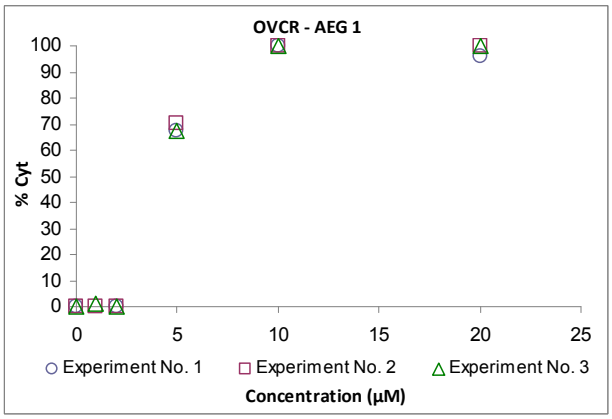


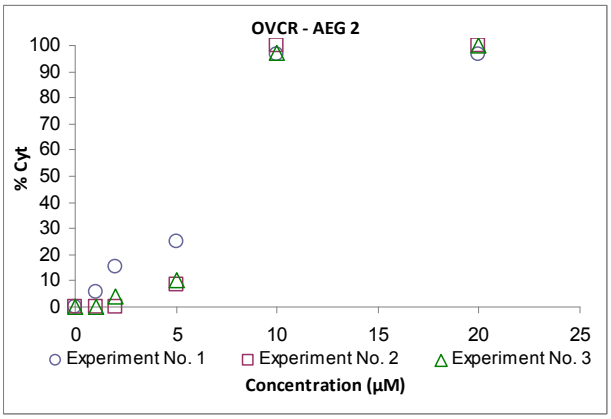


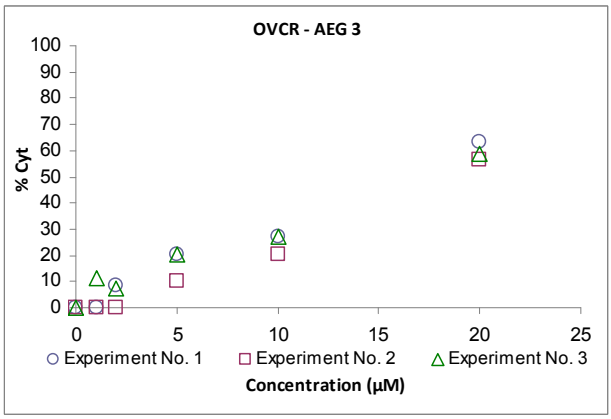
OVCAR-3 IC_{50} Experiments:

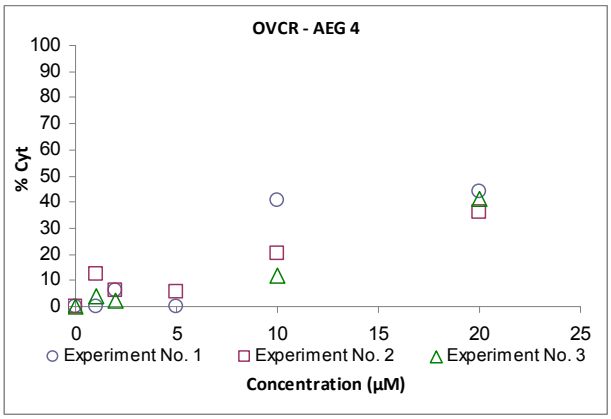




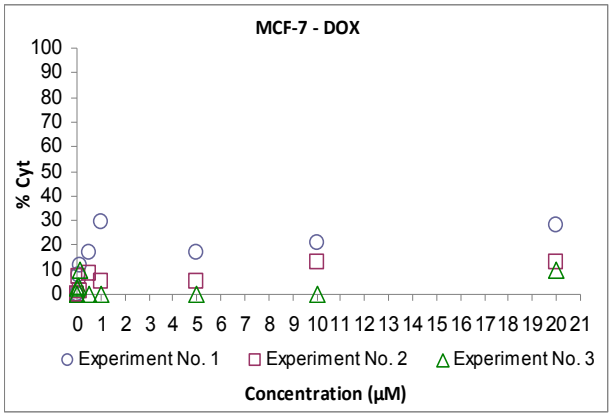


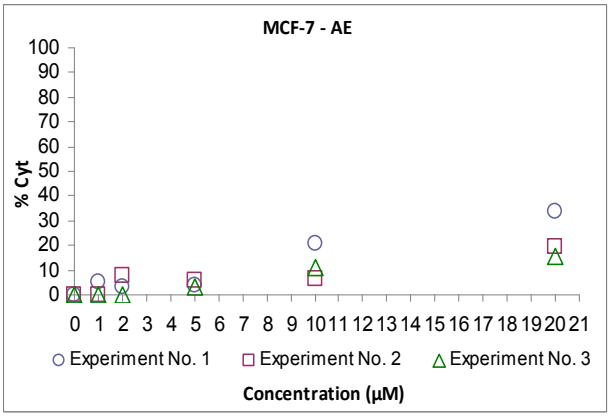


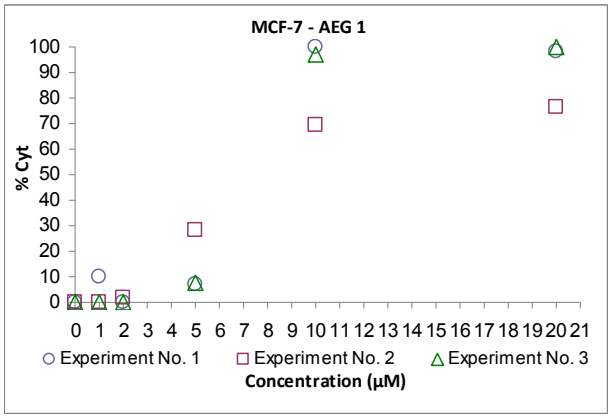


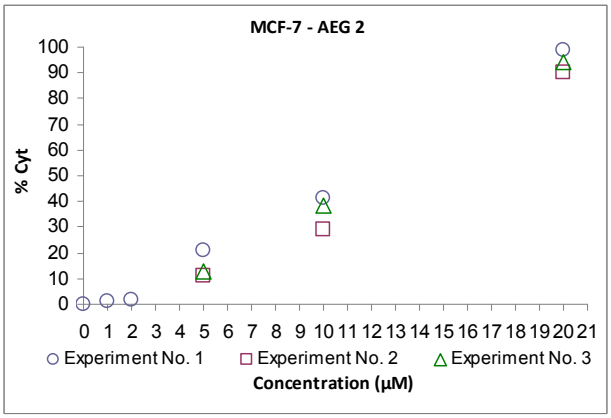


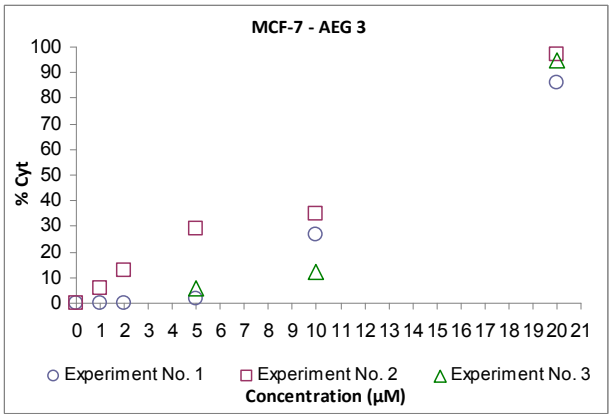
MCF-7 IC_{50} Experiments:

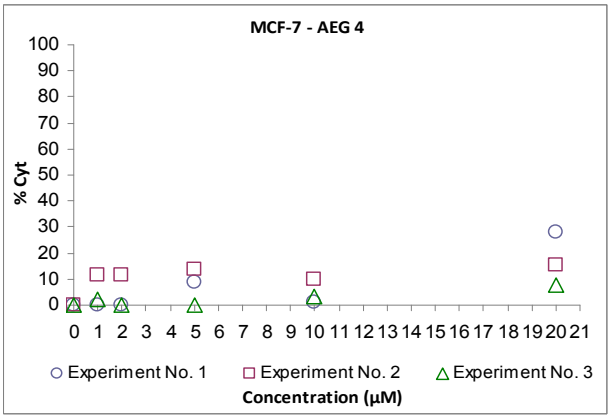




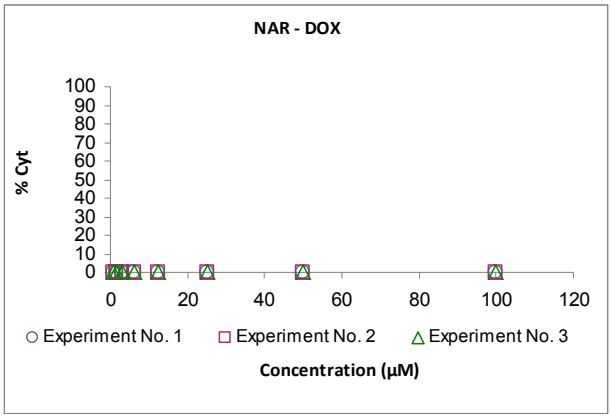


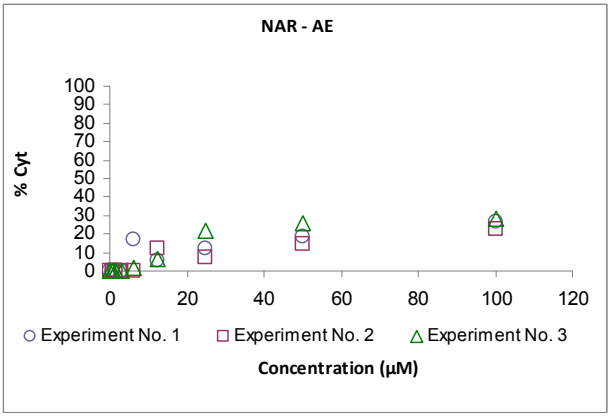


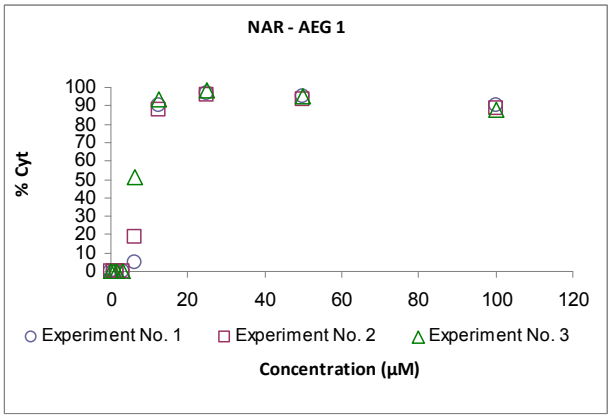


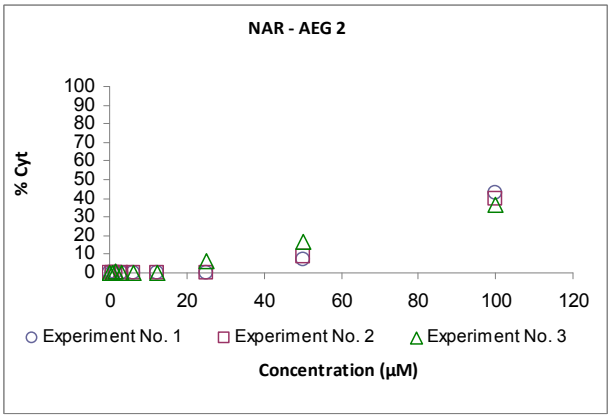


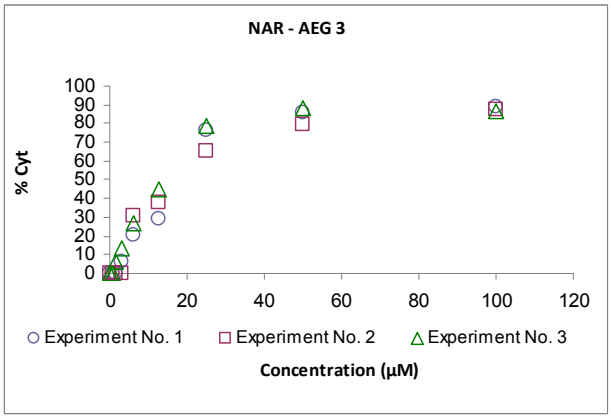
NAR IC_{50} Experiments:

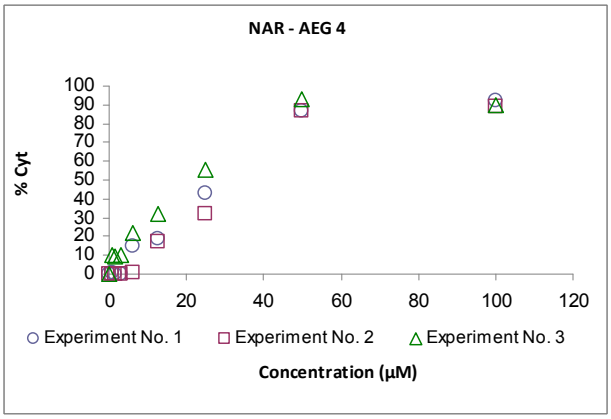




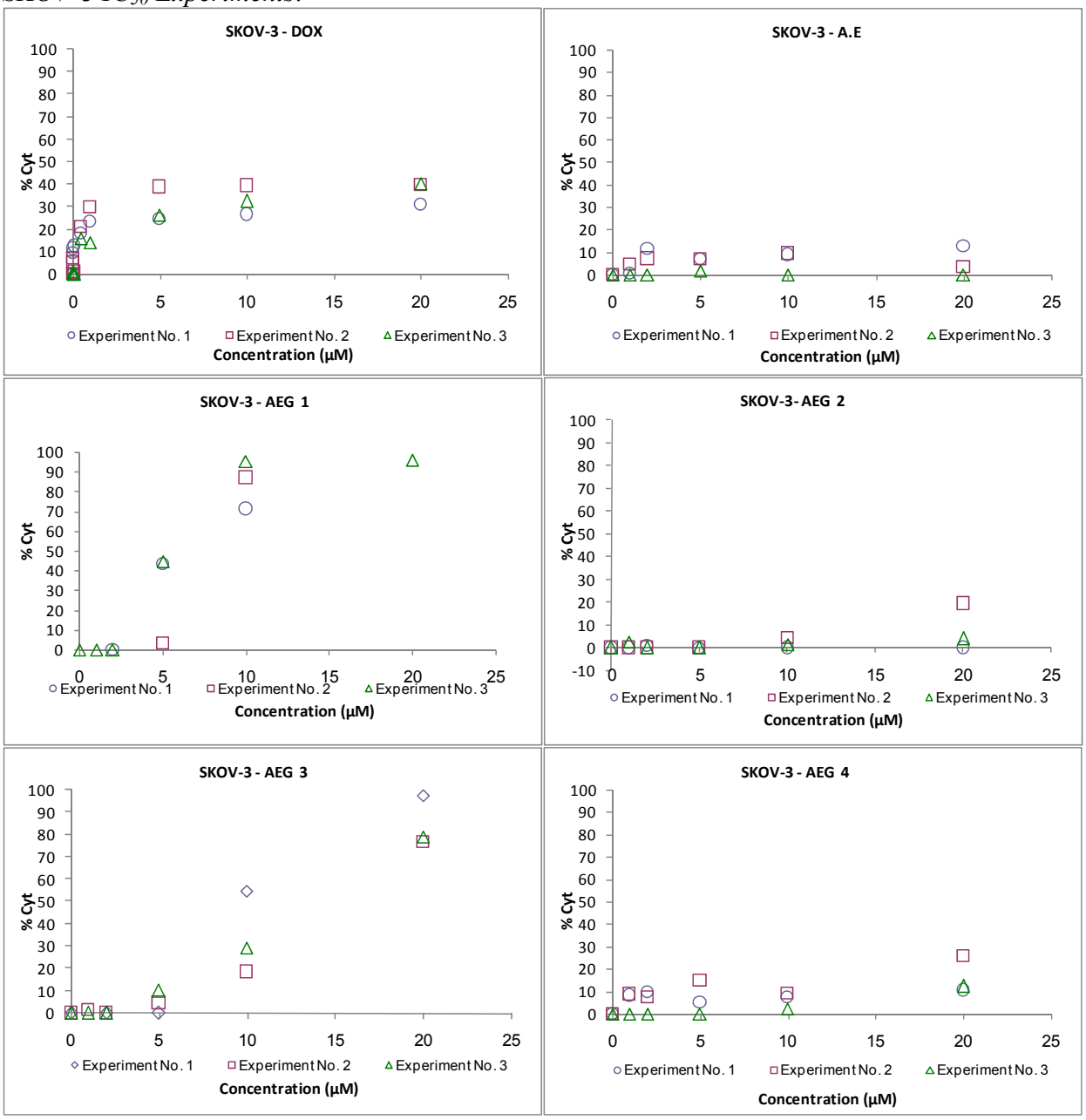








SKOV-3 IC_{50} Experiments:



5. *Supercoiled plasmid DNA unwinding gel electrophoresis protocol*

Eppendorf tubes containing 3 μL supercoiled plasmid PBR322 (0.167 μg/ml), 14 μL tris-HCl-di-natrium-EDTA (TE X1) and 5 μL AE, AEG, or DOX solution were incubated for 15 min at 37ºC. Loading buffer (2 μl) was added, and samples were loaded on a 1% agarose gel in tris-HCl-boric-acid-di-natrium-EDTA (TBE X1). Gels were run for 15 min at 50 V and an additional 225 min at 70 V. The gels were then immersed in a 0.5 mg/ml solution of ethidium bromide, shaken for 20 min, and washed with DDW for 5 min. DNA was visualized by UV illumination.

6. *Trypan blue dye exclusion test of cell viability protocol*

Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 1 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and maintained in a humidified chamber of 95% air 5% CO_2 at 37°C. The cells (2 x 10^5/well) were plated into 6-well microtiter plates and were allowed to adhere before treatment. After 6 hours, 200 μL of AEG 1 or DOX solutions were added at increasing concentrations (0.2, 2, 10 and 20μL) and the cells were incubated for 24 h. Cell viability was determined using Trypan Blue. Cells were incubated with 0.1% trypan blue for 2–5 min and the percentage of dead cells (those, which did not exclude the dye) was determined microscopically as demonstrated for OVCAR-3 cells in Figure 2S. Each concentration was evaluated in triplicate and each experiment was performed three times.

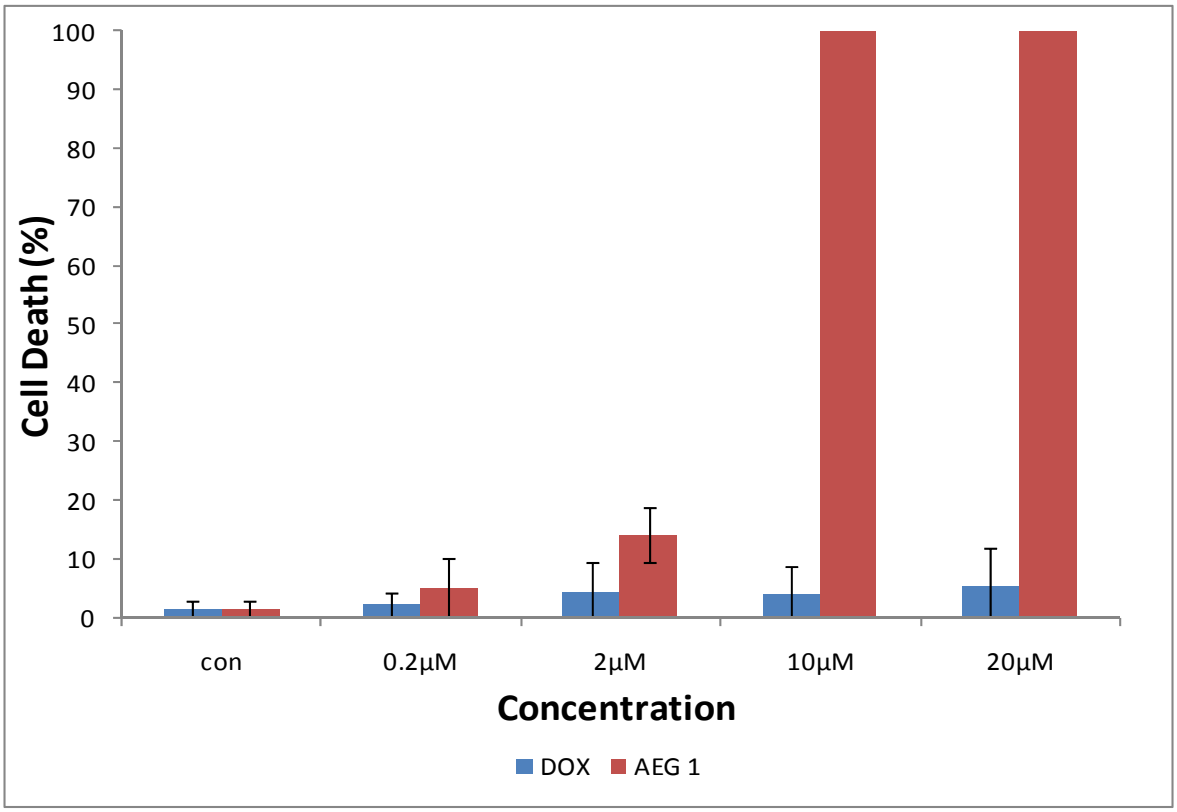
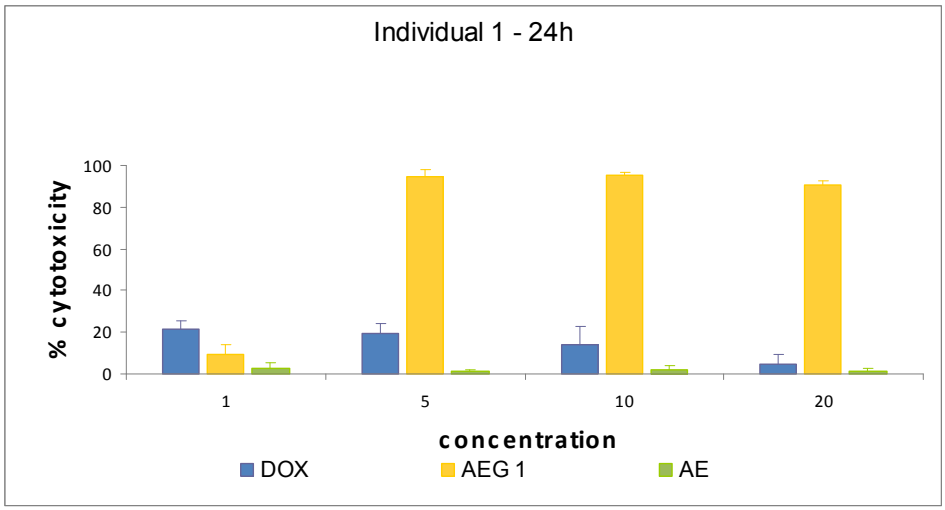


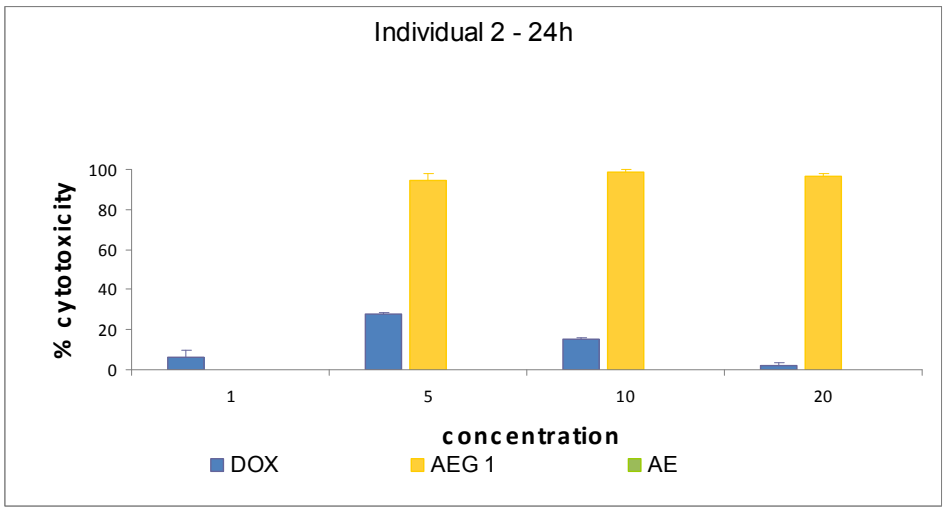
Figure 2s. Trypan blue dye exclusion test of cell viability after 24 hours of incubation of OVCAR-3 cells with AEG-1 and DOX.

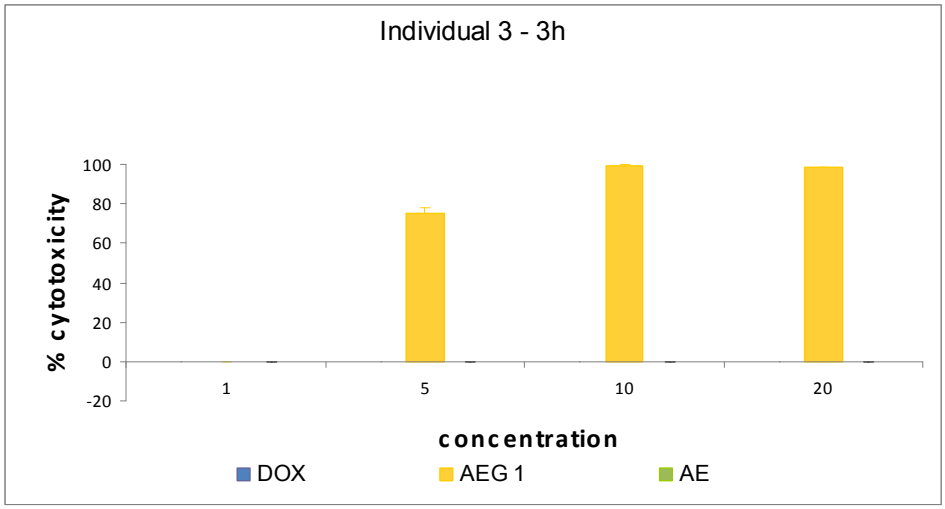
7. *Preparation of peripheral blood cell populations and IC_{50} examination protocol*

Human blood (10 mL) was taken up in a syringe containing 0.2 ml heparin. The blood was diluted with 10 ml RPMI-1640 medium. A new conical tube, containing 18 ml of Ficoll-Paque,

was prepared. The diluted blood was carefully added on the top of the Ficoll-Paque such that the two phases were not mixed. The blood was centrifuged at 2000 RPM for 30 min at room temperature. The cells from the interphase area were transferred to a new conical tube and centrifuged at 2000 RPM for 15 min at 4°C and then resuspended in RPMI. The cells were centrifuged twice at 2000 RPM for 10 min at 4°C and then resuspended in RPMI. Cells were maintained in a humidified chamber of 95% air, 5% CO_2 at 37°C. Cells (35 x 10^3/well) were plated into 96-well microtiter plates and 5 μL of AEG 1, AE, or DOX solutions were added at various concentrations. The cells were incubated for 4 or 24 h. Cell viability was determined using an XTT kit (Biological Industries) as per the manufacturer's instructions. Optical density (OD) was read at 490 nm with a VERSAmax microplate ELISA reader (Molecular Devices). The IC_{50} was the concentration at which the OD value was 50% of the OD value of the untreated cells. Each concentration was evaluated in triplicate and each experiment was performed twice.







8. ***Preparation of red blood cells (RBC) populations and RBC lysis assay***

Fresh blood was obtained from male Wistar rats (~250 g) by cardiac puncture and collected in heperinized tubes. The blood was washed using pre-chilled PBS. PBS was then added to ~10 ml total volume and the samples were centrifuged at 2300 RPM for 10 min at 4°C. The supernatant and the very top 3-4 mm of the pellet were discarded, and the pellet was resuspended in PBS. The cells were washed three times using PBS and centrifuge at 2300 RPM for 10 min at 4°C. After the final centrifugation step, 2 g of the RBCs were transferred to a clean 100-ml volumetric flask add 100 ml pre-chilled PBS was added to yield a 2% w/w RBC stock solution.

The 2% w/w rat RBC solution was incubated with serial dilutions of DOX, AEG **1** and AE for 1 h at 37°C. Negative controls were PBS and dextran (MW 70000), and the positive control was a 1% w/v solution of Triton X100 (100% lysis). Following centrifugation, the supernatant was drawn off and its absorbance measured at 550 nm using a microplate reader (Genios, TECAN). The results were expressed as percentage of hemoglobin released relative to the positive control (Triton X100).

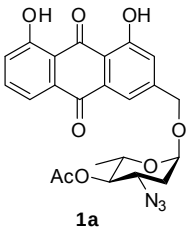
9. ***Confocal microscopy protocol***

Cells (1 x 10^5/well) were plated into 24-well microtiter plates and incubated over coverslips for 24 hours. One of the AEGs, DOX, or AE was added at a concentration of 5 μM. After 2 hours of incubation, the cells were washed three times using PBS (0.5 ml per well) and 0.5 mL

paraformaldehyde (3.7% in PBS) was added. After 15 min at ambient temperature, the cells were washed twice with 0.5 ml PBS 0.5 ml. Cells were shaken at 50 rpm for 5 min after each wash. The plasma membrane of the fixed cells was stained by incubation with 300 μl of $DiIc_{18}(5)$-DS ($4x10^{-4}$g/L) at 4°C. After 20 min, the cells were washed twice with 0.5 ml PBS. Cells were shaken at 50 rpm for 5 min after each wash. Finally, the samples were placed on a microscope slide and fixed with a coverslip using 10 μl of mount. The slides were observed using an Andor Revolution Imaging System equipped with a Yokogawa CSU-X1 Spinning Disk Unit and an iXon 897 back-illuminated EMCCD camera and mounted on a custom made Olympus IX-Upright microscope. Analysis was at Ex/Em = 488/525 nm for DOX and AE glycoside **1** and Ex/Em = 640/685nm for $DiIC_{18}$ (5)-DS.

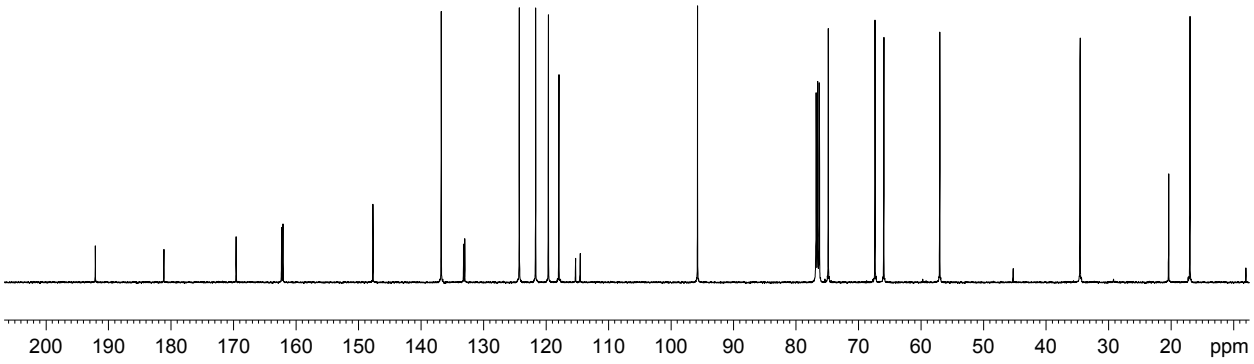
10. *^{1}H and ^{13}C NMR spectra*

^{1}H NMR spectra of **AEG 1a** using 500 MHz

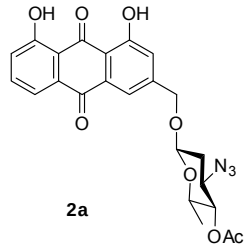


1a

^{13}C NMR spectra of **AEG 1a** using 125.7 MHz

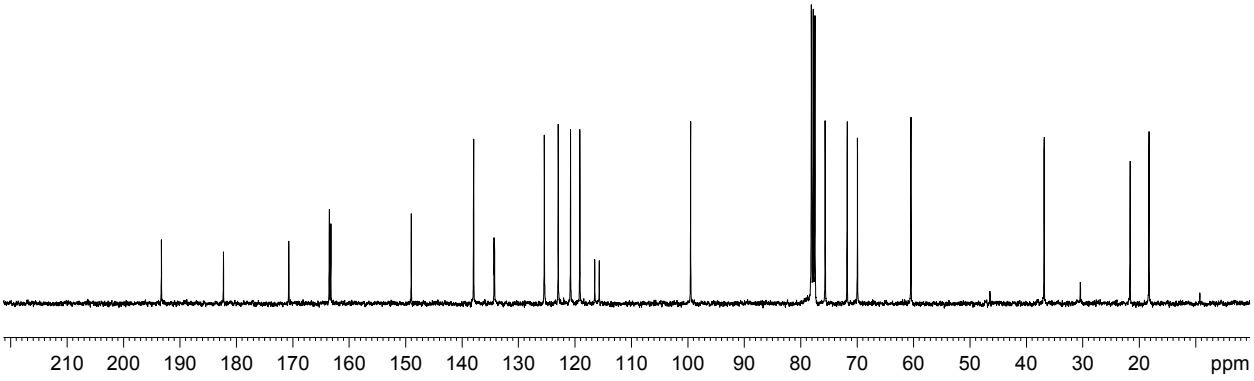


^{1}H NMR spectra of **AEG 2a** using 500 MHz

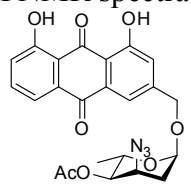


2a

^{13}C NMR spectra of **AEG 2a** using 100.6 MHz

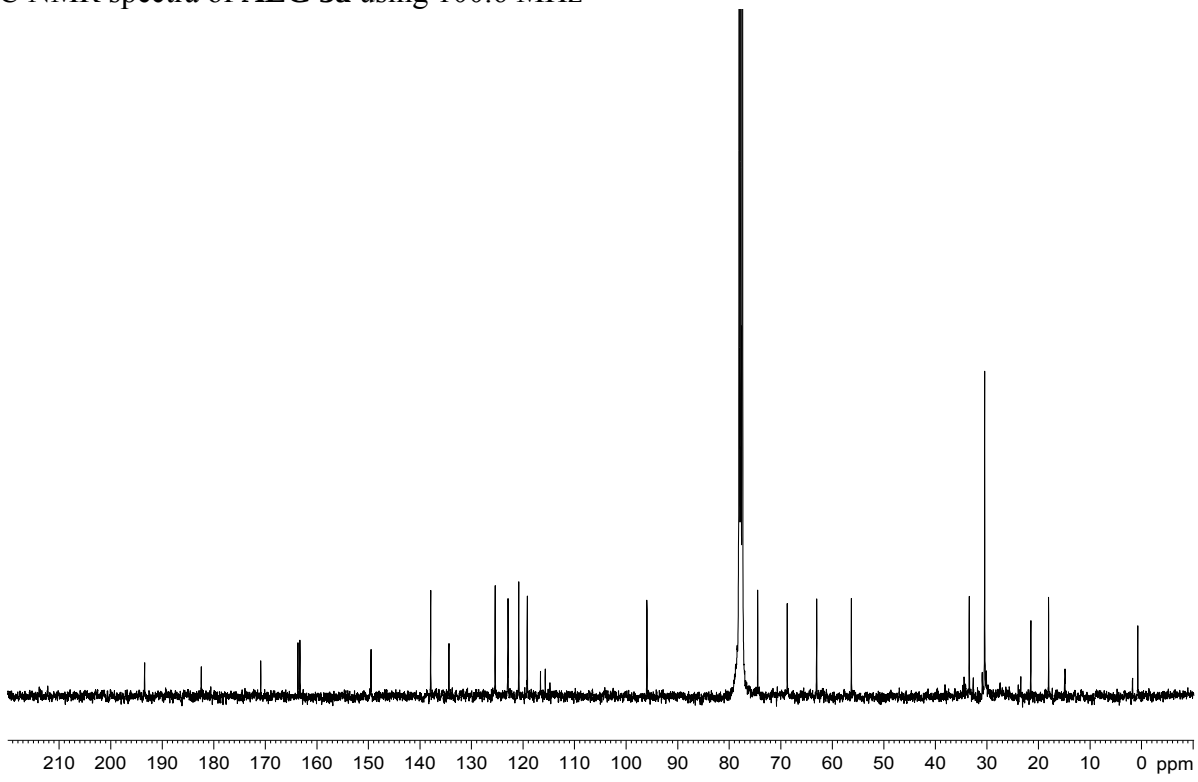


^{1}H NMR spectra of **AEG 3a** using 500 MHz

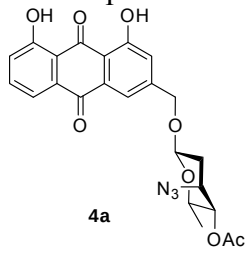


3a

^{13}C NMR spectra of **AEG 3a** using 100.6 MHz

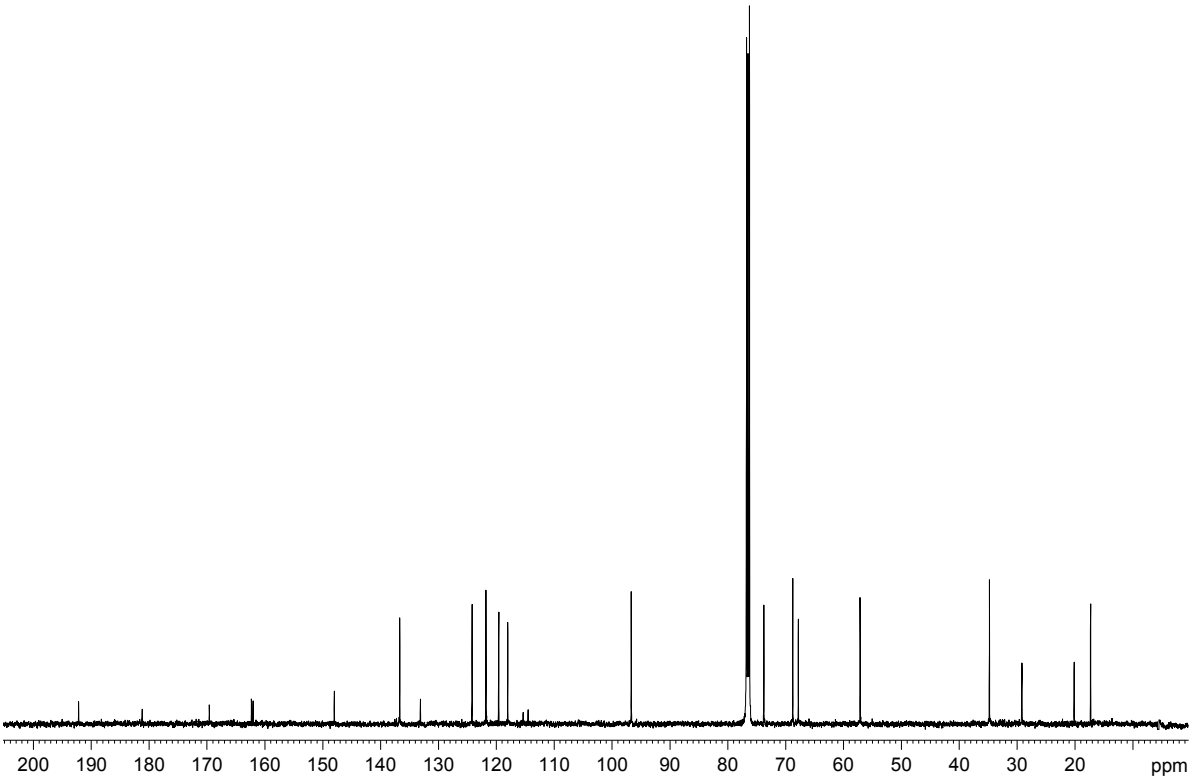


^{1}H NMR spectra of **AEG 4a** using 500 MHz

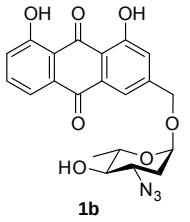


4a

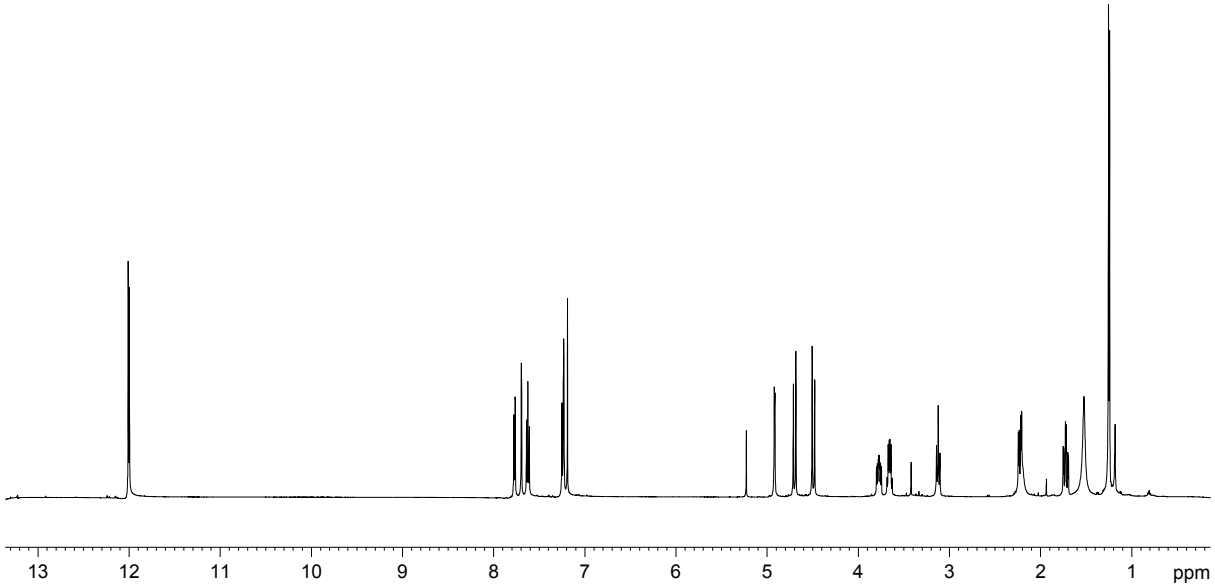
^{13}C NMR spectra of **AEG 4a** using 125.7 MHz



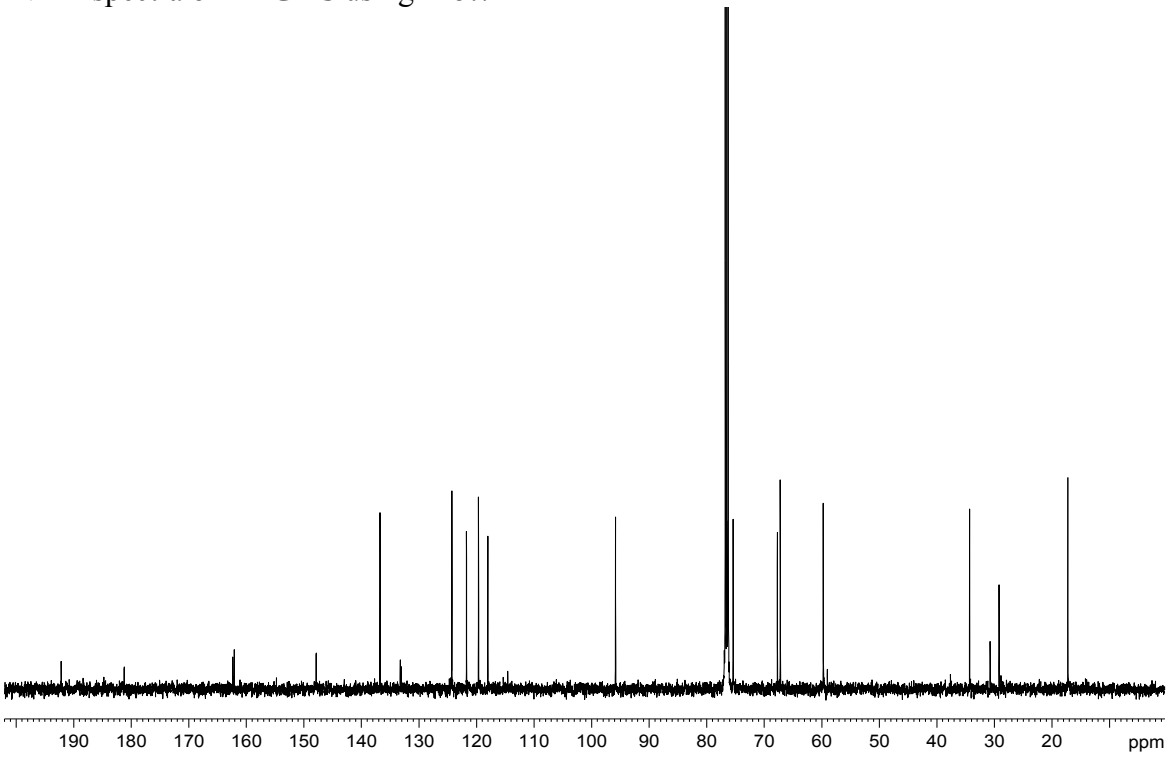
^{1}H NMR spectra of **AEG 1b** using 500 MHz



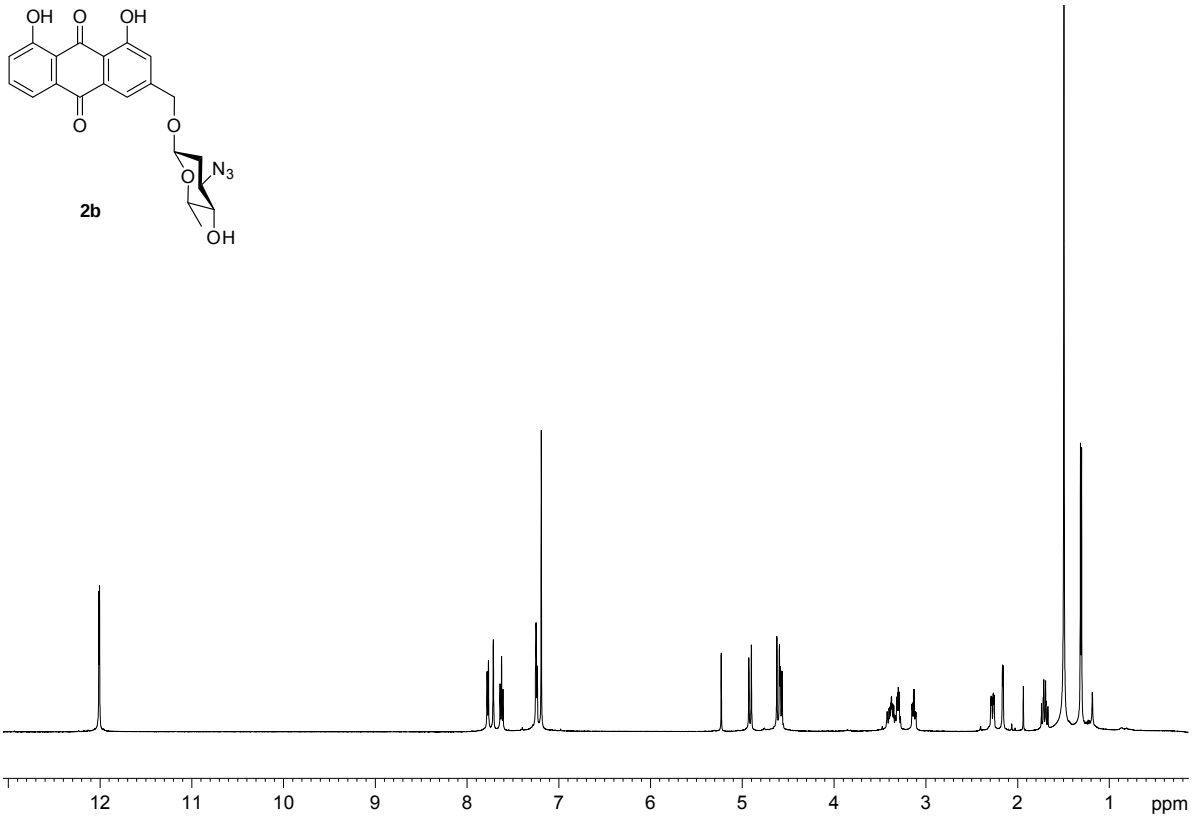
1b



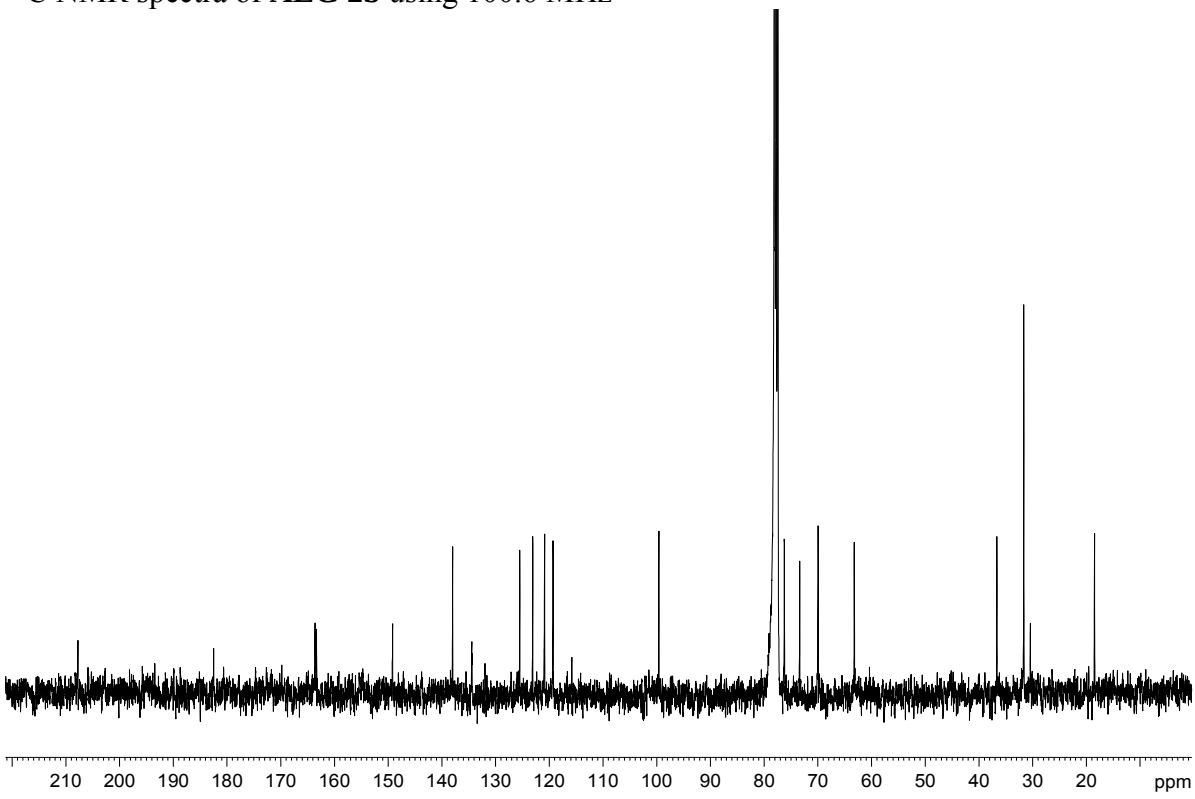
^{13}C NMR spectra of **AEG 1b** using 125.7 MHz



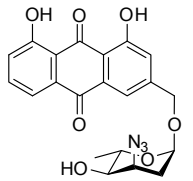
^{1}H NMR spectra of **AEG 2b** using 500 MHz



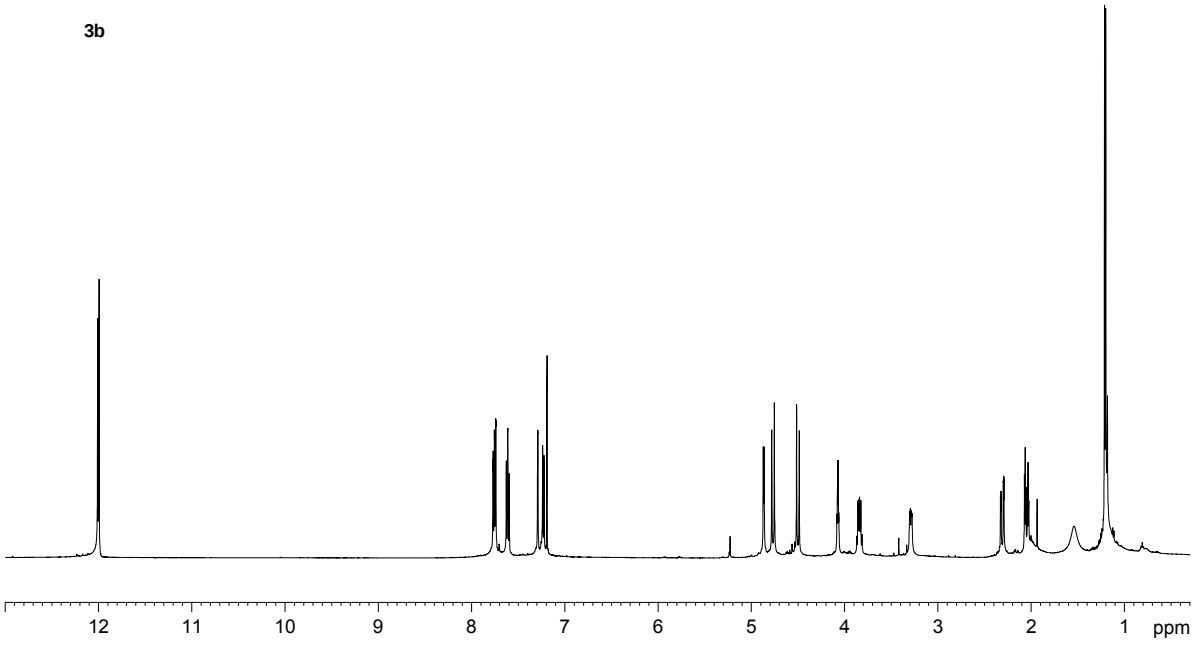
^{13}C NMR spectra of **AEG 2b** using 100.6 MHz



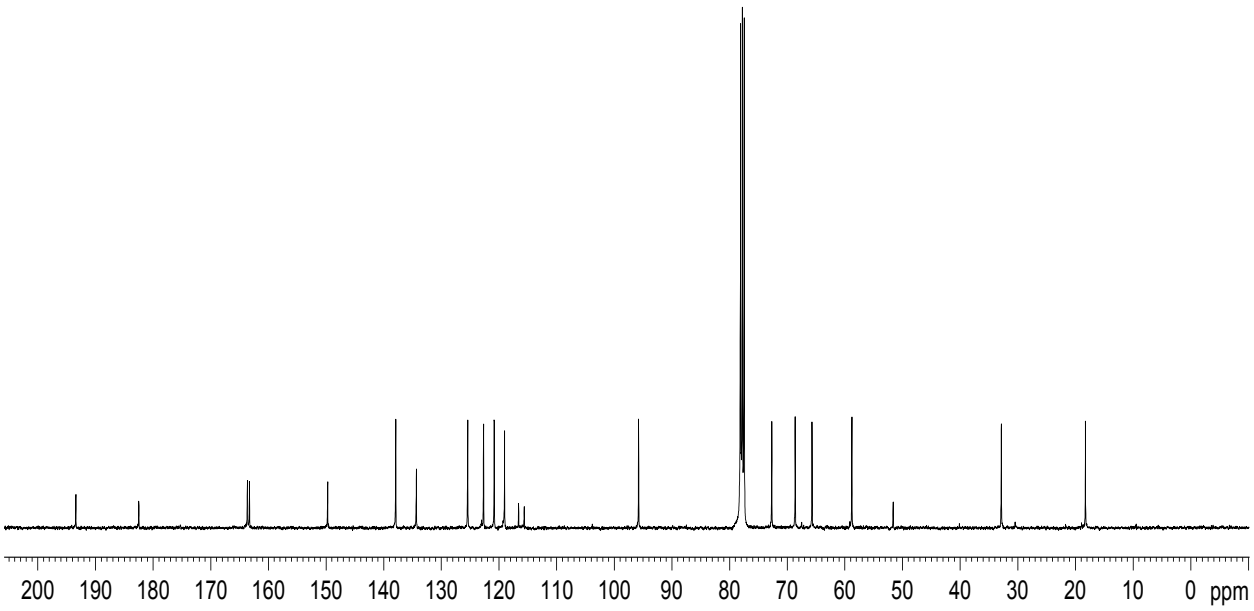
^{1}H NMR spectra of **AEG 3b** using 500 MHz



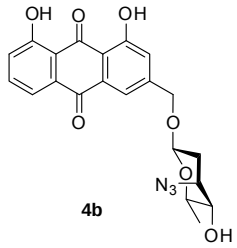
3b



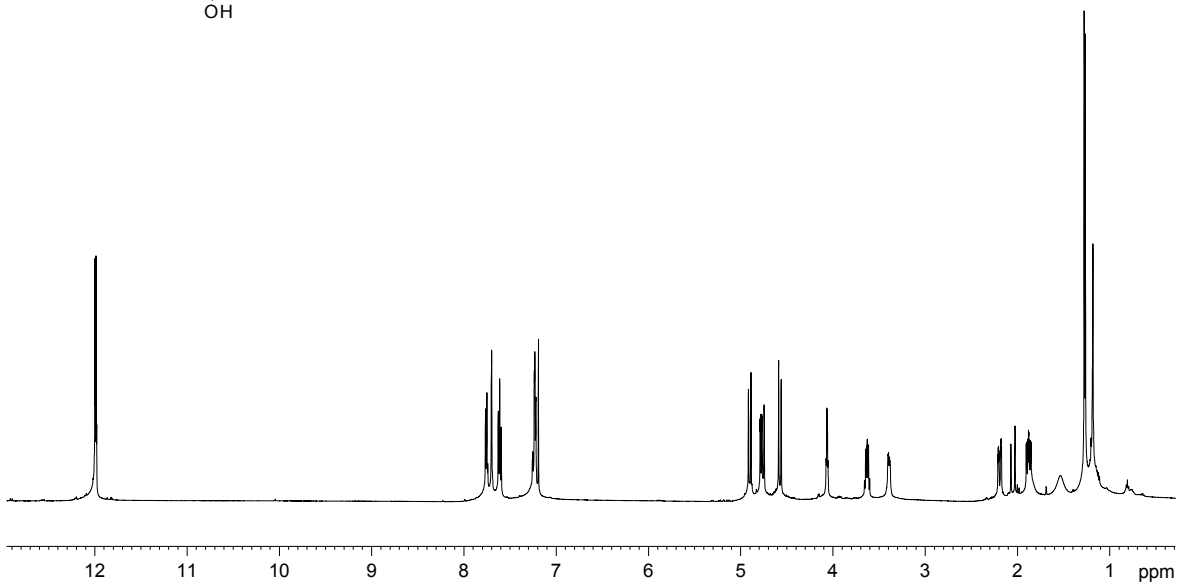
^{13}C NMR spectra of **AEG 3b** using 100.6 MHz



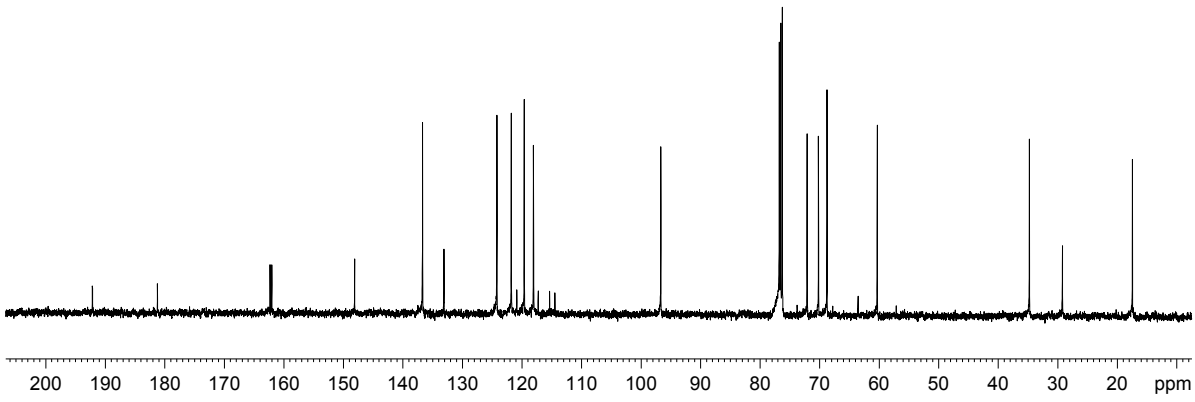
^{1}H NMR spectra of **AEG 4b** using 500 MHz



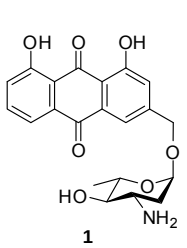
4b



^{13}C NMR spectra of **AEG 4b** using 125.7 MHz

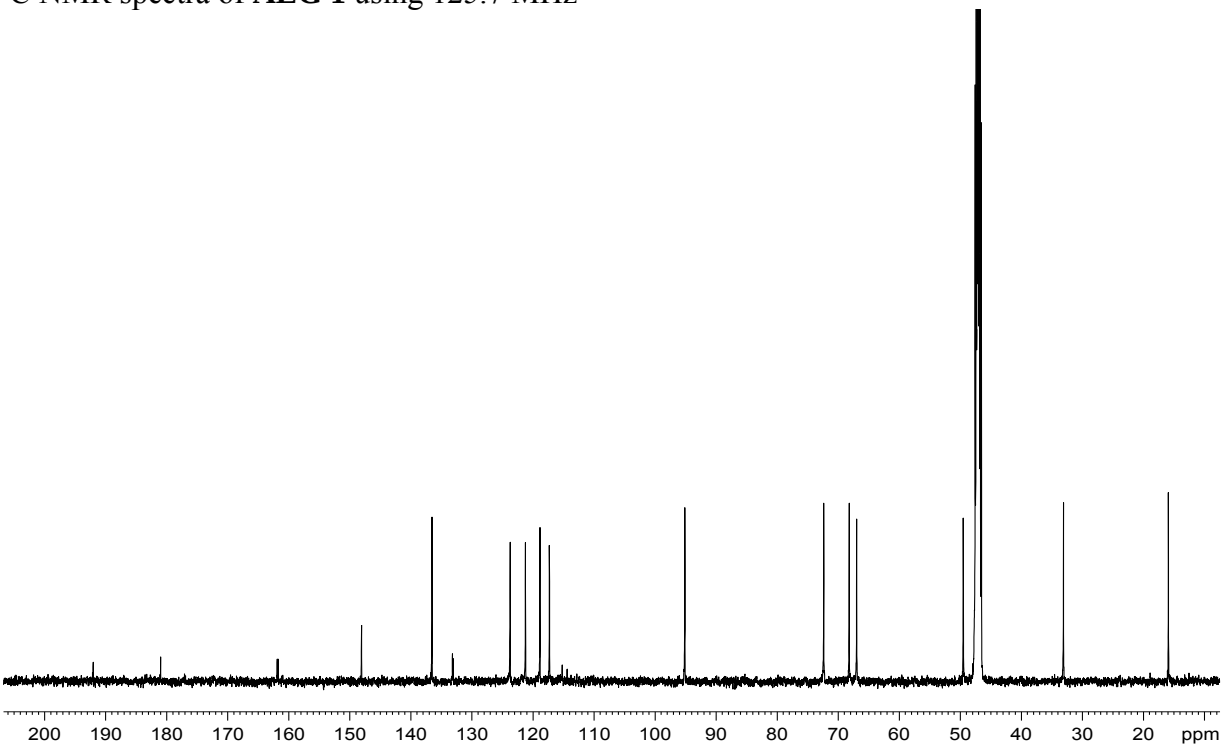


^{1}H NMR spectra of **AEG 1** using 500 MHz

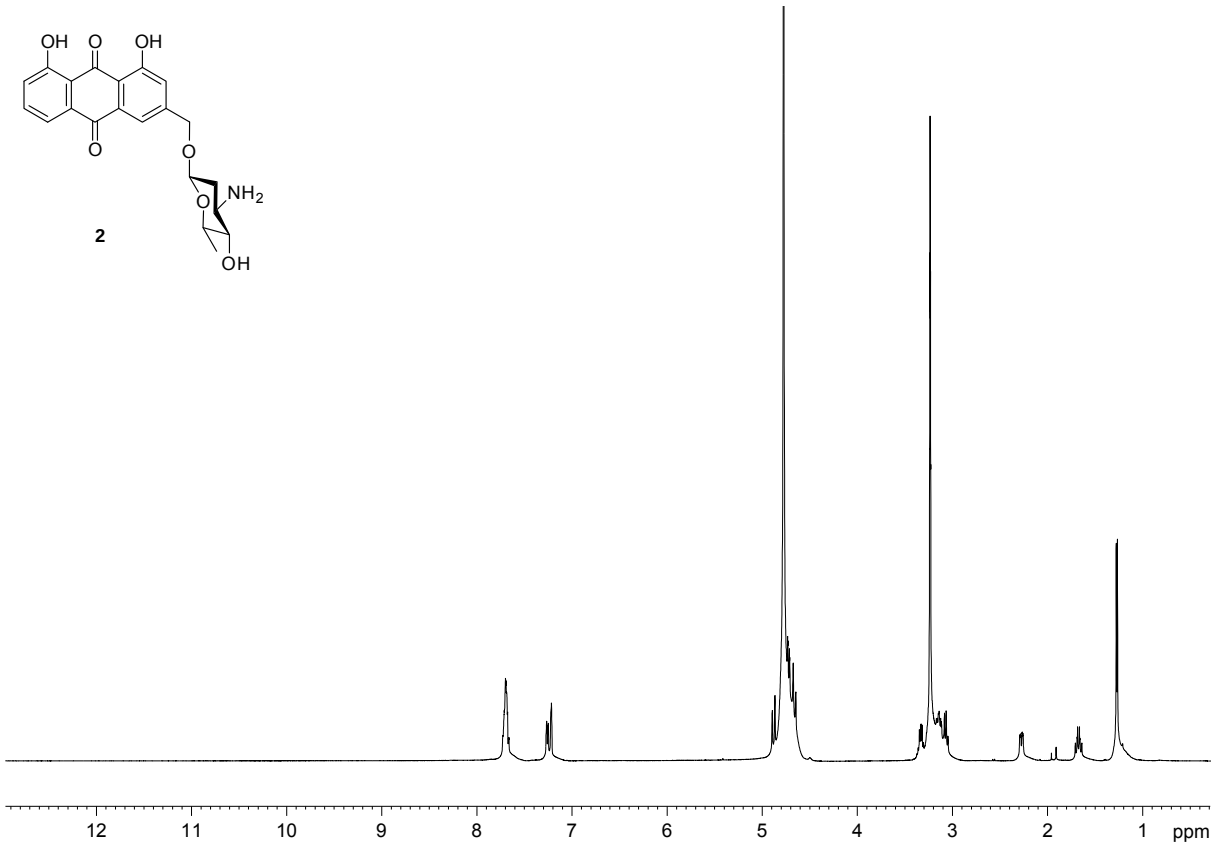


1

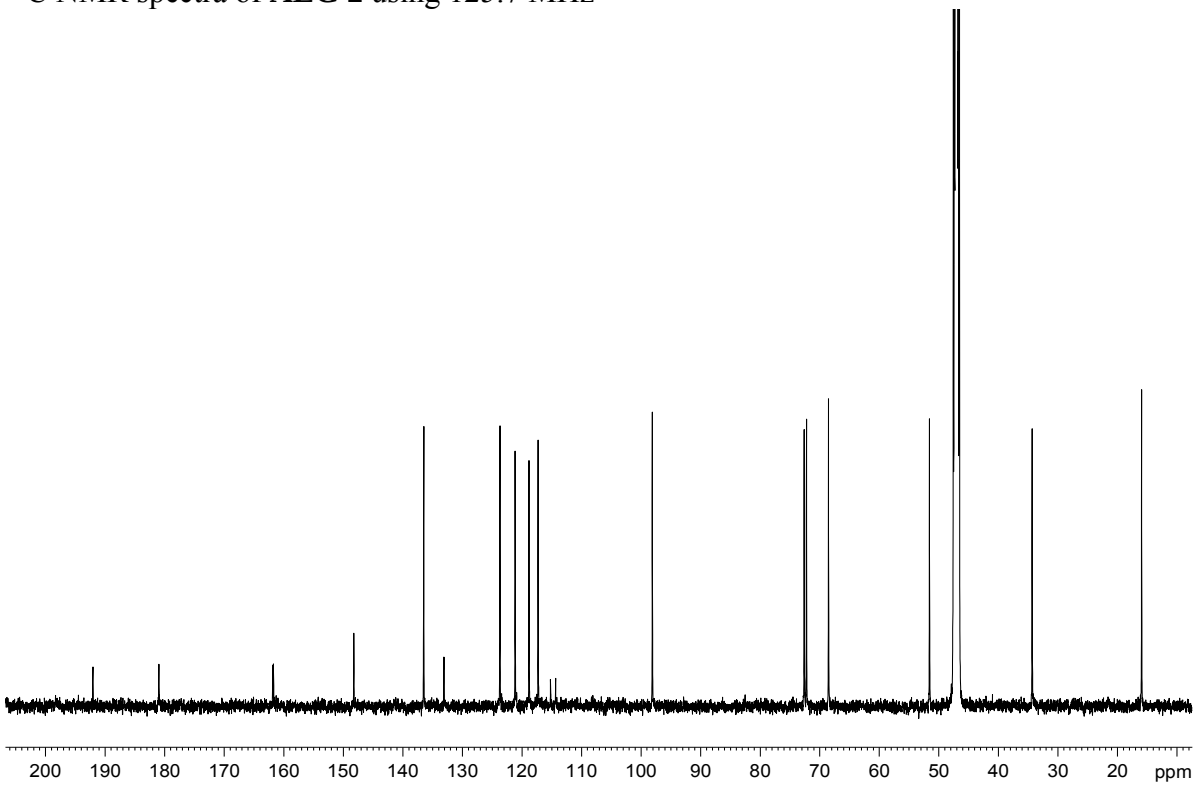
^{13}C NMR spectra of **AEG 1** using 125.7 MHz



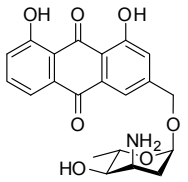
^{1}H NMR spectra of **AEG 2** using 500 MHz



^{13}C NMR spectra of **AEG 2** using 125.7 MHz

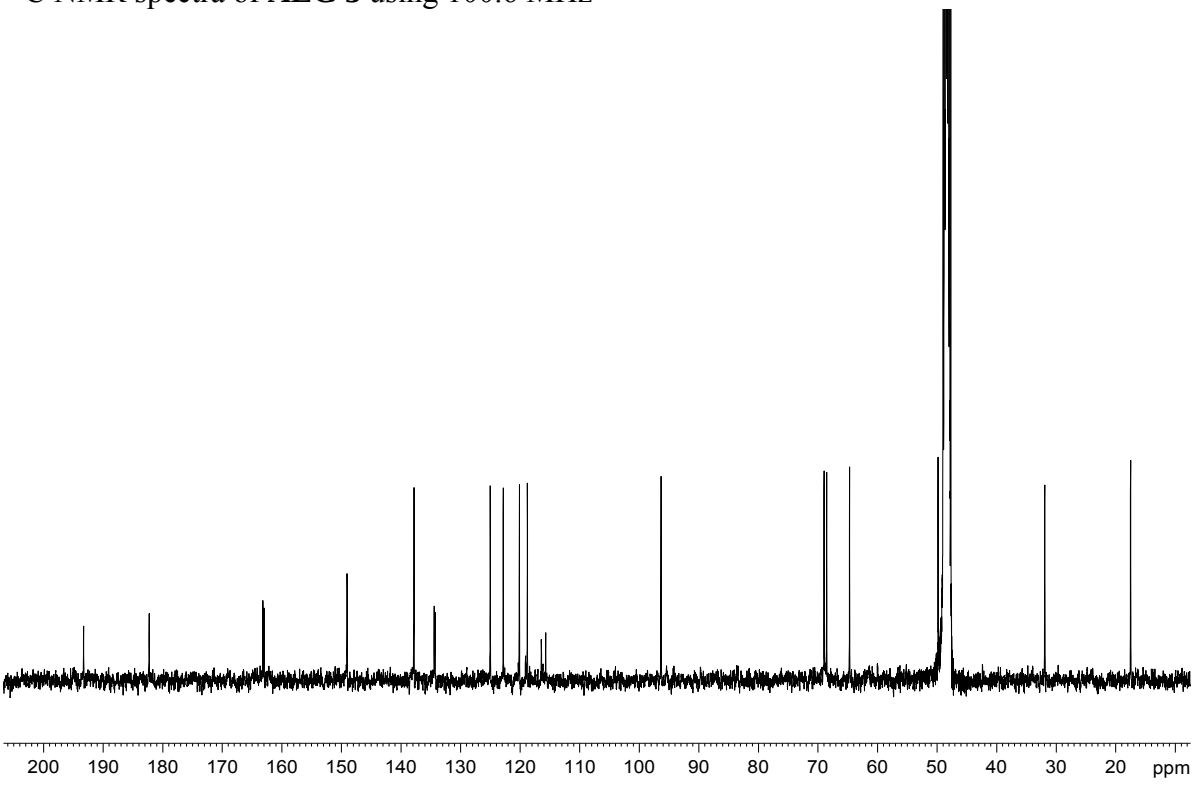


^{1}H NMR spectra of **AEG 3** using 500 MHz

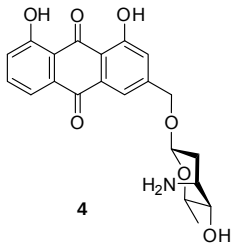


3

^{13}C NMR spectra of **AEG 3** using 100.6 MHz



^{1}H NMR spectra of **AEG 4** using 500 MHz



4

^{13}C NMR spectra of **AEG 4** using 100.6 MHz