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Supporting Information

69451 Weinheim, Germany

Covalent Modification of a Cysteine Residue in the XPB Subunit of the General Transcription Factor TFIIH Through Single Epoxide Cleavage of the Transcription Inhibitor Triptolide**

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Experimental Procedures

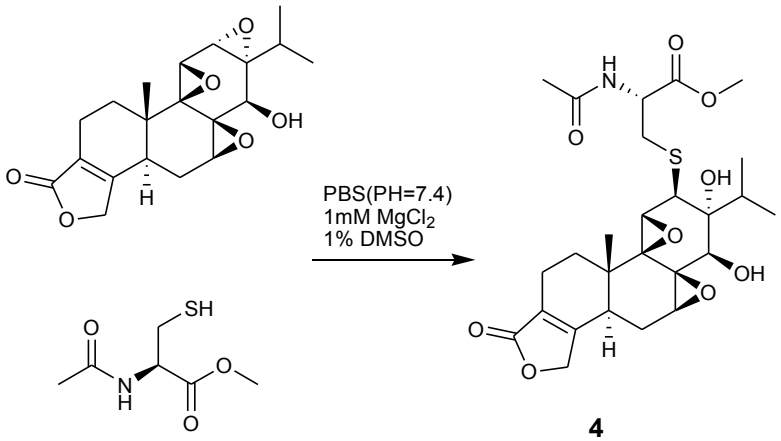
Synthesis of Triptolide analogs

General Experimental

All non-aqueous reactions were performed under a nitrogen atmosphere in oven-dried glassware. Toluene, benzene, diethyl ether, acetonitrile, and methylene chloride were purified by passing through commercially available pre-dried, oxygen-free formulations through activated alumina columns. Tetrahydrofuran was freshly distilled over sodium and benzophenone. Methanol was freshly distilled from Mg turnings. Triethylamine was distilled from calcium hydride and *i*-Pr_2NEt was distilled from potassium hydroxide prior to use. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by LC/MS and TLC. LC/MS was carried out on an ion trap LC/MS using a C18 50 x 2.10 mm 3 micron column, eluting with a solvent gradient of 5:95 → 100 acetonitrile/water in 15.8 min, detecting with UV 250 nm, PDA 190-400 nm and MS ion trap (ionization modes are APCI (+) and (-) or ESI (+) and (-), scan range 100-2100). Thin-layer chromatography (TLC) was carried out on 0.25 mm silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate or an ethanolic solution of para-anisaldehyde and heat as developing agents. Flash column chromatography was performed with 60Å Silica Gel (230-400 mesh) as stationary phase using a gradient solvent system (EtOAc/hexanes as eluent unless indicated otherwise). Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 or 0.50 mm silica gel plates (60F-254). ^1H NMR chemical shifts were measured at

300 or 500 MHz and referenced relative to trace amounts of chloroform (7.26 ppm) and are reported in parts per million. Coupling constants (*J*) are reported in Hertz (Hz), with multiplicity reported following usual convention: s, singlet; d, doublet; t, triplet; q, quadruplet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dddd, doublet of doublet of doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; m, multiplet; br s, broad singlet. ^{13}C NMR spectra were measured at 75 MHz or 125 MHz and referenced relative to residual chloroform (77.16 ppm) and are reported in parts per million (ppm). High resolution mass spectra (HR-MS) were obtained through the Center for Chemical Characterization and Analysis (Texas A&M University) on a mass spectrometer using MALDI (Matrix-assisted laser-desorption ionization) or electrospray ionization.

Synthesis of Analog 4:



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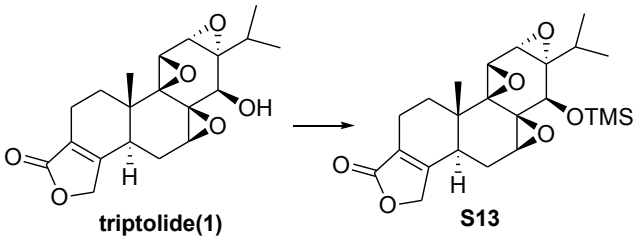
Triptolide(15 mg, 0.0416mmol, 0.1mM, 1.0 equiv) was dissolved in DMSO (4.16mL) and PBS (420ml,including 1mM $MgCl_2$) under argon and to this was added N-acetyl-L-cysteine methyl ester(0.7375g, 4.16mmol, 10mM, 100.0 equiv). After 72hrs, the reaction mixture was extracted with ethyl acetate(5X200ml), and the organic layer was washed with brine (1 x 200 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by HPLC (Agilent pursuit

XRs 5 C18 250X10.0mm, 55%CH_3CN/H_2O) to give Analog **6**(12.4mg, 55%). ^{1}H-NMR(($CD_3)_2CO$,500MHz), δ 4.76(m, 3H), 3.99(d, J=5.5Hz, 1H), 3.73(s, 3H), 3.47(d, J=5.0Hz, 1H), 3.40(d, J=6.0Hz, 1H), 3.31(dd, J=14.0, 5.0Hz, 1H), 3.04(s, 1H), 2.94(dd, J=13.5, 8.0Hz, 1H), 2.85(m, 1H), 2.26(m, 2H), 2.20(m, 1H), 1.95-2.09(m,8H), 1.56(dd, J=12.5, 4.5Hz), 1.39 (m, 1H), 1.09(s, 3H), 1.01(d, J=6.5Hz, 3H), 0.84(d, J=6.5Hz, 3H). ^{13}C-NMR (($CD_3)_2CO$,125MHz), δ 174.3, 172.6, 162.9, 125.7, 79.1, 77.8, 71.4, 70.8, 62.4, 62.1, 60.0, 53.3, 53.2, 48.2, 41.1, 37.2, 34.7, 31.7, 24.3, 23.3, 23.2, 18.4, 16.8, 16.7, 14.8.

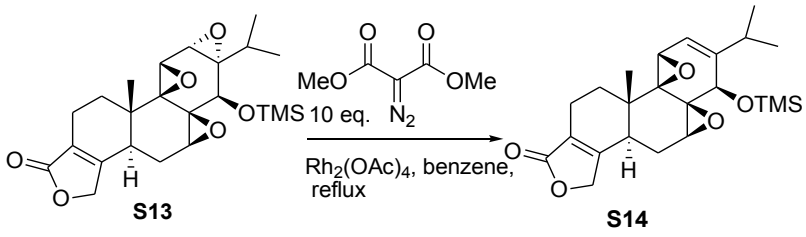
MS (ESI) Calcd for ($M+H^+$) 537.2, Found, 537.3

Synthesis of Analog 5:



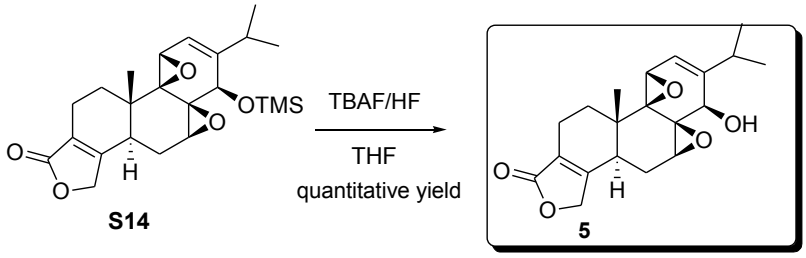
(5bS,6aS,7aR,8R,8aS,9aS,9bS,10aS,10bS)-8a-isopropyl-10b-methyl-8-((trimethylsilyl)oxy)-1,5,5b,6,6a,8,8a,9a,9b,10b-decahydrotris(oxireno)[2',3':4b,5;2'',3'':6,7;2''',3''':8a,9]phenanthro[1,2-c]furan-3(2H)-one (**S13**): Triptolide (**1**, 20.0 mg, 56 μmol) was dissolved in dry CH_2Cl_2 (0.8 mL) and cooled to 0 ^oC under N_2 and Et_3N (23 μL, 166 μmol, 3 equiv) was added. A solution of TMSOTf in CH_2Cl_2 (0.3 mL solution containing 15 μL of TMSOTf) was added dropwise and the mixture was stirred at 0 ^oC for 1 h. Upon completion of the reaction, the mixture was quenched with PBS buffer (0.1 M, 1 mL), extracted with CH_2Cl_2 (2 x 10 mL). The organic layer was washed with brine (5

mL), dried over $MgSO_4$ and concentrated to give the crude material, which was purified by flash chromatography on silica gel (gradient elution, 30→ 40%, EtOAc/hexane) to give desired TMS-protected alcohol **S13** as a white solid (25 mg, ~100% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.66 (d, *J* = 3.0 Hz, 2H), 3.74 (d, *J* = 3.0 Hz, 1H), 3.51 (s, 1H), 3.48 (d, *J* = 3.5 Hz, 1H), 3.22 (d, *J* = 6.0 Hz, 1H), 2.69-2.65 (m, 1H), 2.31-2.26 (m, 1H), 2.22-2.08 (m, 3H), 1.91 (dd, *J* = 15.0, 13.5 Hz, 1H), 1.58 (dd, *J* = 13.0, 4.5 Hz, 1H), 1.26-1.23 (m, 1H), 1.21-1.15 (m, 1H), 1.06 (s, 3H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.82 (d, *J* = 6.5 Hz, 3H), 0.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 160.5, 125.7, 74.1, 70.1, 65.3, 63.8, 61.1, 60.2, 55.1, 55.0, 40.6, 35.9, 29.6, 27.7, 23.9, 17.6, 17.2, 16.7, 13.6, 0.48 (3C); HRMS (ESI+) calcd. for $C_{23}H_{33}O_6Si$ ($M+H^+$) 433.2046, found 433.2040.



(5aS,5bS,6aS,9R,9aR,10aS,11aS)-8-isopropyl-5a-methyl-9-((trimethylsilyl)oxy)-4,5,5a,6a,9,10a,11,11a-octahydrobis(oxireno)[2',3':4b,5;2'',3'':8a,9]phenanthro[1,2-c]furan-3(1H)-one (**S14**): Tris-epoxide **S13** (8 mg, 20.4 μmol) was mixed with $Rh_2(OAc)_4$ catalyst (0.9 mg, 2 μmol, 0.1 equiv), vacuum dried for 5 min and purged with N_2. Dry benzene (1.3 mL) was added to give a clear green solution, which was heated to 80 °C. Diazo reagent (32 mg, 204 μmol, 10 equiv) was dissolved in anhydrous benzene (0.65 mL) and added slowly (0.1 mL/h via syringe pump) to the reaction mixture at 80 °C. Upon complete addition, the mixture was stirred at 80 °C for 2 h before slowly being cooled down

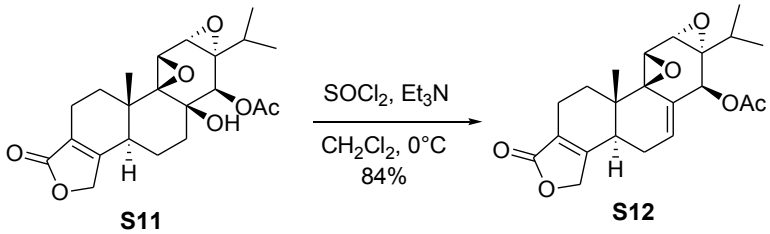
to ambient temperature and stirred overnight. The mixture was filtered through a short silica gel pad, rinsed with 40% EtOAc/Hexanes (5 x 1 mL). The filtrate was concentrated to give the crude material, which was purified by flash chromatography on silica gel (gradient elution, 20→ 50%, EtOAc/hexane) followed by prep. TLC purification (eluent: 2% MeOH/ CH_2Cl_2) to give desired des-epoxy **S14** as a colorless oil (1.0 mg, 13% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 (d, *J* = 4.5 Hz, 1H), 4.68 (br s, 2H), 3.53 (d, *J* = 4.5 Hz, 1H), 3.51 (s, 1H), 3.28 (d, *J* = 5.5 Hz, 1H), 2.68 (d, *J* = 13.0 Hz, 1H), 2.34 (p, *J* = 7.0 Hz, 1H), 2.30-2.15 (m, 1H), 2.17-2.01 (m, 1H), 2.09 (dt, *J* = 15.0, 6.0 Hz, 1H), 1.99 (t, *J* = 13.5 Hz, 1H), 1.71 (dd, *J* = 12.0, 5.0 Hz, 1H), 1.23-1.18 (m, 1H), 1.13 (s, 3H), 1.08 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.16 (s, 9H); HRMS (ESI+) calcd. for $C_{23}H_{32}O_5SiNa$ ($M+Na^+$) 439.1917, found 439.1904.



(5aS,5bS,6aS,9R,9aS,10aS,11aS)-9-hydroxy-8-isopropyl-5a-methyl-4,5,5a,6a,9,10a,11,11a-octahydrobis(oxireno)[2',3':4b,5;2'',3'':8a,9]phenanthro[1,2-c]furan-3(1H)-one (**5**): A stock solution of 1M TBAF/HF in THF was prepared by mixing 1M TBAF/THF (1 mL) with HF/Py (70 wt%, 25 μL). 24 μL of such solution was further diluted in 1 mL THF and 0.2 mL of diluted solution (containing 4.8 μmol of TBAF and 4.8 μmol HF) was mixed with **S14** (0.5 mg, 1.2 μmol). The mixture was stirred at ambient temperature for ~7 h until full consumption of starting material as evidenced by TLC. The reaction mixture was then extracted with CH_2Cl_2 (5 x 2 mL). The organic layer was washed with sat. NH_4Cl (2 mL), sat.

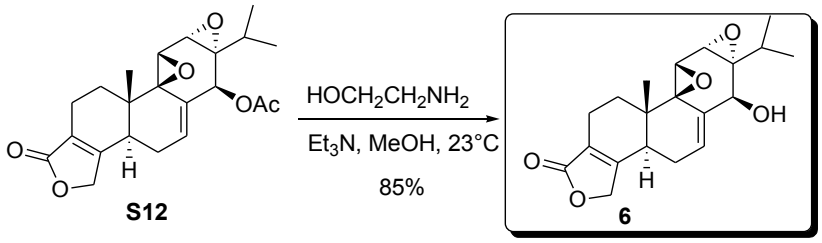
$NaHCO_3$ (2 mL) and brine (2 mL), dried over $MgSO_4$ and concentrated to give the crude material, which was purified by flash chromatography on silica gel (gradient elution, 30→ 70%, EtOAc/hexane) to give the desired des-epoxy alcohol **5** as a colorless oil (0.4 mg, 99% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 (d, *J* = 4.5 Hz, 1H), 4.68 (br s, 2H), 3.70 (d, *J* = 4.5 Hz, 1H), 3.40 (d, *J* = 5.5 Hz, 1H), 3.30 (d, *J* = 11.5 Hz, 1H), 2.69-2.64 (m, 1H), 2.52 (d, *J* = 11.5 Hz, 1H), 2.43 (p, *J* = 7.0 Hz, 1H), 2.36-2.32 (m, 1H), 2.18-2.13 (m, 2H), 2.03 (t, *J* = 13.5 Hz, 1H), 1.67 (dd, *J* = 12.0, 5.0 Hz, 1H), 1.28-1.22 (m, 1H), 1.18 (s, 3H), 1.10 (d, *J* = 7.0 Hz, 3H), 1.04 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{20}H_{25}O_5$ ($M+H^+$) 345.1702, found 345.1715.

Synthesis of Analog 6:



(5bS,8S,8aR,9aS,9bS,10aR,10bS)-8a-isopropyl-10b-methyl-3-oxo-2,3,5,5b,6,8,8a,9a,9b,10b-decahydro-1H-bis(oxireno)[2',3':4b,5;2'',3'':6,7]phenanthro[1,2-c]furan-8-yl acetate (**S12**): Acetate **S11** (2.0 mg, 5.0 μmol) was dissolved in dry CH_2Cl_2 (0.3 mL) and cooled to 0 °C and then Et_3N (21 μL, 149 μmol, 30 equiv) was added. A stock solution of $SOCl_2$ in CH_2Cl_2 was prepared by mixing $SOCl_2$ (18 μL) in CH_2Cl_2 (1 mL) and 100 μL of this solution (containing 1.8 μL of $SOCl_2$, 25 μmol, 5 equiv) was added to the reaction mixture at 0 °C. The mixture was stirred at 0 °C for 1 h. Upon completion of the reaction, the mixture was quenched with sat. $NaHCO_3$ solution (2 mL), extracted with CH_2Cl_2 (4 x 2 mL). The organic layer was

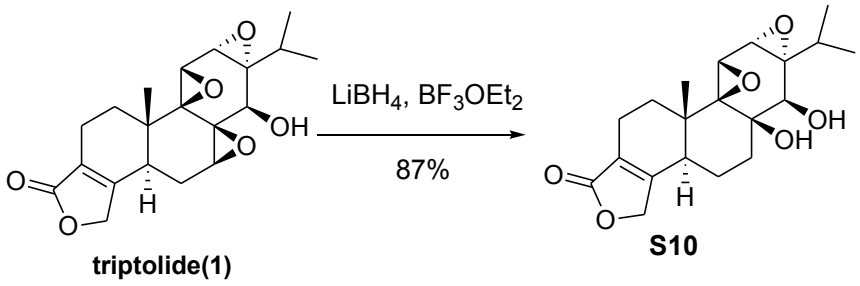
washed with brine (2 mL), dried over $MgSO_4$ and concentrated to give the crude material, which was purified by flash chromatography on silica gel (gradient elution, 20→ 66%, EtOAc/hexane) to give desired alkene **S12** as a colorless oil (1.6 mg, 84% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.32 (dd, *J* = 5.5, 1.5 Hz, 1H), 5.76 (s, 1H), 4.71 (d, *J* = 19.0 Hz, 1H), 4.67 (d, *J* = 18.5 Hz, 1H), 3.72 (d, *J* = 3.0 Hz, 1H), 3.55 (d, *J* = 3.5 Hz, 1H), 2.78 (d, *J* = 12.0 Hz, 1H), 2.37 (d, *J* = 16.0 Hz, 1H), 2.27 (dt, *J* = 17.5, 5.5 Hz, 1H), 2.19-2.09 (m, 2H), 2.11 (s, 3H), 1.98 (p, *J* = 7.0 Hz, 1H), 1.64 (dd, *J* = 13.0, 5.0 Hz, 1H), 1.30 (dt, *J* = 12.5, 6.5 Hz, 1H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.90 (s, 3H), 0.88 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{22}H_{27}O_6$ ($M+H^+$) 387.1808, found 387.1815.



(5bS,8S,8aR,9aS,9bS,10aR,10bS)-8-hydroxy-8a-isopropyl-10b-methyl-5,5b,6,8,8a,9a,9b,10b-octahydro-1H-bis(oxireno)[2',3':4b,5;2'',3'':6,7]phenanthro[1,2-c]furan-3(2H)-one (**6**): Alkene **S12** (4.6 mg, 11.9 μmol) was dissolved in MeOH (0.3 mL) and mixed with Et_3N (100 μL, 717 μmol, 60 equiv). 2-hydroxylethylamine (120 μL, 1994 μmol, 168 equiv) was added to the reaction mixture and the resulting solution was stirred at ambient temperature for 24 h. Upon completion of the reaction, the mixture was extracted with CH_2Cl_2 (3 x 5 mL). The organic layer was washed with sat. NH_4Cl solution (2 mL), brine (2 mL), dried over $MgSO_4$ and concentrated to give the crude material, which was purified by flash chromatography on silica gel (gradient elution, 20→ 66%,

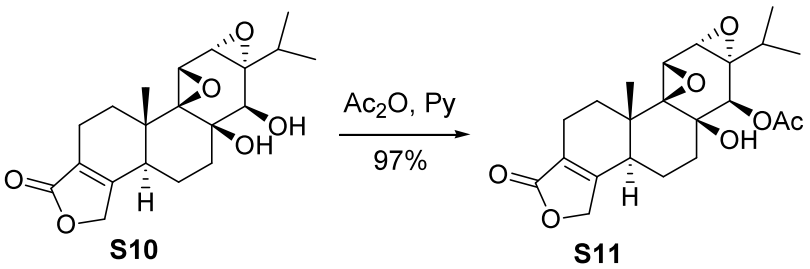
EtOAc/hexane) to give desired alcohol **6** as a white solid (2.6 mg, 63% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.06 (dd, *J* = 5.5, 2.0 Hz, 1H), 4.71 (br s, 2H), 4.34 (dd, *J* = 12.0, 1.0 Hz, 1H), 3.83 (d, *J* = 3.0 Hz, 1H), 3.52 (dd, *J* = 3.0, 1.0 Hz, 1H), 2.89 (d, *J* = 12.0 Hz, 1H), 2.81 (d, *J* = 12.0 Hz, 1H), 2.39 (ddd, *J* = 18.0, 4.0, 2.0 Hz, 1H), 2.29 (dd, *J* = 12.5, 5.0 Hz, 1H), 2.27-2.25 (m, 1H), 2.21-2.12 (m, 2H), 1.61 (dd, *J* = 12.0, 5.0 Hz, 1H), 1.30 (dt, *J* = 12.0, 6.0 Hz, 1H), 1.05 (d, *J* = 7.0 Hz, 3H), 0.98 (s, 3H), 0.91 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{20}H_{25}O_5$ ($M+H^+$) 345.1702, found 345.1691.

Synthesis of Analog 7:

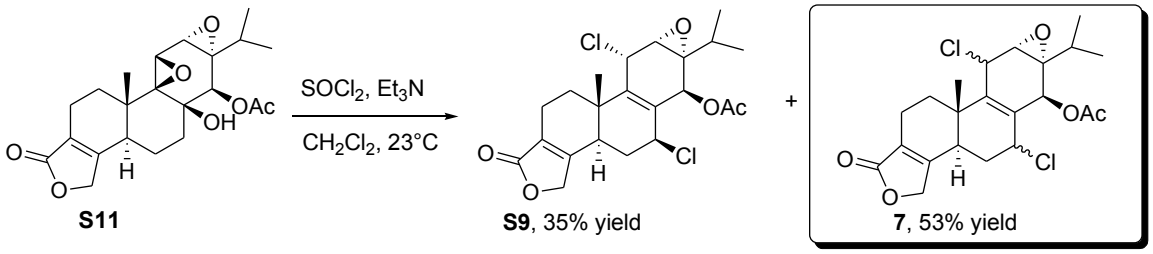


(5bS,7aR,8S,8aR,9aS,9bS,10aS,10bS)-7a,8-dihydroxy-8a-isopropyl-10b-methyl-5,5b,6,7,7a,8,8a,9a,9b,10b-decahydro-1H-bis(oxireno)[2',3':4b,5;2'',3'':6,7] phenanthro[1,2-c]furan-3(2H)-one (**S10**): To a solution of triptolide (**1**, 9 mg, 28 μmol) in dry THF (2 mL) was added a $LiBH_4$ solution (2 N, 28 μL, 56 μmol) followed by BF_3•Et_2O (neat solution, 7 μL, 56 μmol). The mixture was stirred under N_2 at ambient temperature for 3 h. Upon completion of the reaction, the mixture was quenched with *aq*. HCl solution (1 N, 1 mL) and stirred for additional 10 min. The mixture was extracted with CH_2Cl_2 (3 x 5 mL), the organic layer was washed with $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography on silica gel (gradient elution, 20→ 66%, EtOAc/hexane) to give diol **S10** as a colorless oil (7.1 mg, 78% yield): ^{1}H

NMR (500 MHz, $CDCl_3$) δ 4.76-4.67 (m, 2H), 3.84 (d, *J* = 2.0 Hz, 1H), 3.83 (d, *J* = 3.5 Hz, 1H), 3.55 (d, *J* = 12.0 Hz, 1H), 3.44 (dd, *J* = 3.0, 1.0 Hz, 1H), 2.57 (d, *J* = 12.5 Hz, 1H), 2.53-2.50 (m, 1H), 2.36-2.19 (m, 3H), 2.15 (tq, *J* = 13.0, 2.0 Hz, 1H), 1.96 (dq, *J* = 13.0, 3.5 Hz, 1H), 1.67-1.57 (m, 3H), 1.28 (dt, *J* = 11.5, 7.0 Hz, 1H), 1.23 (s, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.85 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{20}H_{27}O_6$ ($M+H^+$) 363.1808, found 363.1815.



(5bS,7aR,8S,8aS,9aS,9bS,10aS,10bS)-7a-hydroxy-8a-isopropyl-10b-methyl-3-oxo-2,3,5,5b,6,7,7a,8,8a,9a,9b,10b-dodecahydro-1H-bis(oxireno)[2',3':4b,5;2'',3'':6,7] phenanthro[1,2-c]furan-8-yl acetate (**S11**): A stock solution of DMAP in CH_2Cl_2 was prepared by dissolving 2 mg DMAP in 16 mL of CH_2Cl_2. 0.8 mL of this DMAP/CH_2Cl_2 solution was then added to **S10** (7.0 mg, 25 μmol) to give a clear solution, to which Ac_2O (4.7 μL, 50 μmol) and Et_3N (14 μL, 100 μmol) were added respectively. The mixture was stirred at ambient temperature for 24 h to give 70% conversion. The mixture was then concentrated and the residue was purified by flash chromatography on silica gel (gradient elution, 20→ 66%, EtOAc/hexane) to give acetate **S11** as colorless oil (5.0 mg, 63% yield): ^1H NMR (500 MHz, $CDCl_3$) δ 5.20 (s, 1H), 4.71 (dd, *J* = 28.0, 17.0 Hz, 2H), 3.83 (d, *J* = 3.0 Hz, 1H), 3.44 (d, *J* = 3.0 Hz, 1H), 2.54 (d, *J* = 12.5 Hz, 1H), 2.35-2.32 (m, 1H), 2.27-2.23 (m, 1H), 2.26 (d, *J* = 2.0 Hz, 1H), 2.20 (s, 3H), 2.12-2.05 (m, 1H), 1.95-1.83 (m, 2H), 1.61-1.54 (m, 3H), 1.31 (dt, *J* = 12.0, 6.5 Hz, 1H), 1.17 (s, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.79 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{22}H_{29}O_7$ ($M+H^+$) 405.1913, found 405.1924.



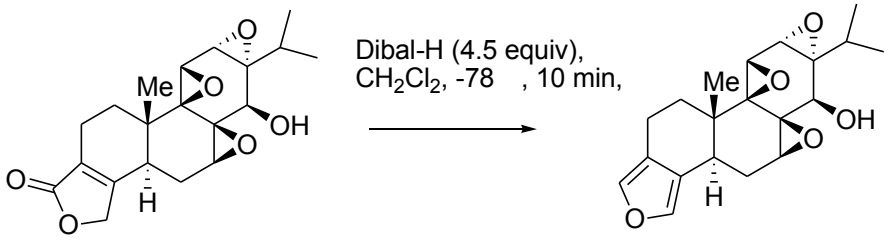
Acetate **11** (1.6 mg, 4.4 μmol) was dissolved in dry CH_2Cl_2 (0.4 mL) and Et_3N (58 μL, 417 μmol, 95 equiv) was added. A stock solution of $SOCl_2$ in CH_2Cl_2 was prepared by mixing $SOCl_2$ (65 μL) in CH_2Cl_2 (1 mL) and 100 μL of this solution (containing 6.5 μL of $SOCl_2$, 88.8 μmol, 20 equiv) was added to the reaction mixture and stirred at ambient temperature for 20 min. Upon completion of the reaction as determined by TLC, the mixture was quenched with sat. $NaHCO_3$ solution (1 mL), extracted with CH_2Cl_2 (2 x 5 mL). The organic layer was concentrated, and the residue was purified by flash chromatography on silica gel (gradient elution, 20→ 66%, EtOAc/hexane) to give dichloride **9** (0.6 mg, 35% yield) and diastereomeric dichloride **7** (0.9 mg, 53% yield) both as colorless oils.

(3bR,5S,6S,6aR,7aR,8S,8bS)-5,8-dichloro-6a-isopropyl-8b-methyl-1-oxo-1,3,3b,4,5,6,6a,7a,8,8b,9,10-dodecahydrooxireno[2',3':6,7]phenanthro[1,2-c]furan-6-yl acetate (**S9**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.04 (s, 1H), 4.97 (d, *J* = 3.0 Hz, 1H), 4.80 (d, *J* = 3.5 Hz, 1H), 4.80 (d, *J* = 17.5 Hz, 1H), 4.65 (d, *J* = 18.0 Hz, 1H), 3.53 (br s, 1H), 3.08 (d, *J* = 12.5 Hz, 1H), 2.52 (d, *J* = 15.5 Hz, 1H), 2.36-2.33 (m, 1H), 2.28 (dt, *J* = 14.5, 5.0 Hz, 1H), 2.18-2.12 (m, 2H), 2.14 (s, 3H), 2.11-2.05 (m, 1H), 1.63-1.57 (m, 1H), 1.15 (s, 3H) 1.05 (d, *J* = 7.0 Hz,

3H), 0.95 (d, *J* = 7.0 Hz, 3H) ; HRMS (ESI+) calcd. for $C_{22}H_{27}Cl_2O_5$ ($M+H^+$) 441.1236, found 441.1250.

5,8-dichloro-6a-isopropyl-8b-methyl-1-oxo-1,3,3b,4,5,6,6a,7a,8,8b,9,10-dodecahydrooxireno[2',3':6,7]phenanthro[1,2-c]furan-6-yl acetate (**7**) (stereochemistry at C7,C11 unconfirmed): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (s, 1H), 4.93 (d, *J* = 4.5 Hz, 1H), 4.81 (d, *J* = 16.5 Hz, 1H), 4.70 (d, *J* = 4.0 Hz, 1H), 4.65 (d, *J* = 17.0 Hz, 1H), 3.52 (d, *J* = 4.5 Hz, 1H), 3.30 (d, *J* = 12.5 Hz, 1H), 2.53 (d, *J* = 20.5 Hz, 1H), 2.32 (dd, *J* = 13.0, 6.0 Hz, 1H), 2.21 (dt, *J* = 13.0, 4.5 Hz, 1H), 2.17 (s, 3H), 2.26-2.14 (m, 2H), 2.07-2.02 (m, 2H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.96 (s, 3H), 0.88 (d, *J* = 7.0 Hz, 3H); HRMS (ESI+) calcd. for $C_{22}H_{27}Cl_2O_5$ ($M+H^+$) 441.1236, found 441.1224.

Synthesis of Analog 8 (Furan-Triptolide):



8

Triptolide (20 mg, 0.0555mmol, 1.0 equiv) was dissolved in anhydrous CH_2Cl_2 (2mL) and cooled to -78 °C under argon and to this was added Dibal-H (1.0 M in Hexanes, 250µL, 0.250mmol, 4.5 equiv) dropwise. The solution was stirred for an additional 10 minutes at -78 °C, then the reaction mixture was partitioned between EtOAc(30 mL) and 1M Rochelle's salt (20 mL). The biphasic mixture was warmed to room temperature and stirred vigorously for 1 hr, and the aqueous layers were separated and the organic layer was washed with H_2O (3 x 10 mL) and

brine (1 x 15 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography (Hexanes-20% EtOAc/Hexanes) to give Analog **2** (7.5mg, 39%). ^{1}H-NMR ($CDCl_3$,400MHz) δ 7.14(S, 2H), 3.93(d,J=2.8Hz,1H), 3.53(d, J=2.4Hz,1H), 3.41(d, J=10.8Hz,1H),3.36(d,J=5.2Hz,1H), 2.82(d, J=10.8Hz, 1H), 2.70(dd, J=12.0, 8.0Hz, 1H), 2.58(dd, J=16.0, 8.0Hz, 1H), 2.44 (m, 1H), 2.28 (m,1H), 2.00 (m,1H), 1.47 (dd, J=12.0,4.0Hz, 1H),1.19(m, 1H),1.10(s,3H), 1.02(d, J=6.8Hz, 3H), 0.89(d, J=6.8Hz, 3H). ^{13}C-NMR($CDCl_3$, 100MHz), δ 137.7, 137.4, 124.3, 119.0, 73.8, 67.0, 65.7, 60.7, 60.6, 56.9, 54.7, 37.6, 35.9, 31.0, 28.0, 26.1, 17.7, 16.8, 15.5, 12.9. **MS** (ESI) Calcd. for ($M+H^+$) 345.2, Found, 345.2

Materials. Triptolide was purchased from various commercial sources including Sigma, and ^{3}H-triptolide was prepared by AmBios Labs, Inc. with specific activity of 4.0 Ci/mmol. General chemicals were purchased from Sigma and Fisher. ^{32}γ-ATP, [^{3}H]-thymidine and [^{3}H]-uridine were purchased from Perkin Elmer.

Cell proliferation assay. HEK293T cells were seeded at 10,000 cells per well in 96-well plates and cultured in DMEM plus 10% FBS at 37 ^{0}C with 5% CO_2. Twenty four hours after seeding, drugs were added at indicated concentrations and incubation was continued for an additional 24 h (unless indicated otherwise). An aliquot of 1 μCi of [^{3}H]-thymidine (Perkin Elmer) was added per well and incubation was continued for an additional 6 h (unless indicated otherwise). Cells were harvested onto a Printed Filtermat A glass fiber filter (Perkin Elmer) using Tomtec Harvester 96 Mach III and filters were immersed in Betaplate Scint (Perkin Elmer) scintillation fluid, followed by scintillation counting on 1450 Microbeta JET (Perkin Elmer).

Determination of triptolide modification site on XPB. Six hundred microliters of HeLa nuclear extract containing 4 mg of total protein was incubated with 10 μM triptolide in 1.2 ml of 10 mM HEPES (pH 8.0), 50 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 0.1 mM EDTA, 0.5 mM DTT and 50 μM PMSF for 1 h at 30 ^{0}C. Fifteen micrograms (15 μl) of affinity purified rabbit anti-XPB antibody (A301-337A, Bethyl Laboratories) was added and the mixture was incubated for 1 hour at 4 ^{0}C. The mixture was added to 100 μl of Dynabeads Protein A (100.01D, Invitrogen, storage solution removed) and further mixed by rotation for 15 min at room temperature. The supernatant was aspirated and the beads were washed 3 times with 500 μl PBST and one time with 500 μl PBS. The beads were resuspended in 40 μl of sample buffer, boiled for 5 min and subjected to SDS-PAGE. The protein band of interest was exercised and in-gel digested. The resulting tryptic digest was dissolved in 3 μL of HPLC solvent A (0.1% formic acid in water, v/v) and injected into an Eksigent NanoLC-1D plus HPLC system (Eksigent Technologies) equipped with an in-house packed 360 μm OD × 75 μm ID reversed phase C12 column. Peptides were eluted from 5% to 70% HPLC solvent B (0.1% formic acid in acetonitrile, v/v) in solvent A at a flow rate of 200 nl/min using a 2-hour cycle. Mass spectrometric analysis was performed on a LTQ Orbitrap Velos mass spectrometer (ThermoFisher Scientific). Precursor ion masses were acquired in the Orbitrap mass spectrometer with a resolution of 60,000 at *m/z* 400, followed by data-dependent MS/MS fragmentation of the 20 most intense precursor ions in the linear ion trap mass spectrometer. PTMap software was used for unrestrictive protein post-translational modification analysis.[5]

[^{3}H]-triptolide binding to recombinant his-XPB or TFIIH. Wild type or mutant his-XPB (469 ng) was incubated with or without 50 μM cold triptolide in 40 μl binding buffer [20 mM Tris (pH 7.9), 4 mM $MgCl_2$, 1 μM ATP, 100 μg/ml BSA, and 100 nM Adenosine Major Late Promoter (AdMLP)] for 1 h at 30 ^{0}C. One micromole per liter of [^{3}H]-triptolide (4 Ci/mmol) was added and the mixture was incubated for 1 h 30 min at 30^{0}C. Samples were boiled in sample buffer and subjected to 12% SDS-PAGE. Wild type or mutant TFIIH (70 nM) was incubated with or without 200 μM cold triptolide in 30 μl binding buffer [20 mM Hepes (pH 7.9), 4 mM $MgCl_2$, 1 mM TCEP, indicated concentrations of ATP and AdMLP for 1 h at RT. One micromole per liter of [^{3}H]-triptolide (4 Ci/mmol) was added and the mixture was incubated for 1 h 30 min at RT. Samples were boiled in sample buffer and subjected to 8% Phosphate SDS-PAGE. After electrophoresis, the gel was soaked in En3hance solution (Perkin Elmer) according to manufacturer instructions and exposed to pre-flashed x-ray film for 2 or 3 weeks prior to development.

Binding-dialysis-activity recovery assay. A 50-μl reaction mixture including 20 mM Hepes (pH 7.9), 4 mM $MgCl_2$, 1mM TCEP, 1mg/ml BSA, 20 nM TFIIH and 10/100 μM triptolide or its analogs was incubated for 2 h at RT. Then the reactions were dialyzed in 2 x 250ml dialysis buffer [20 mM Hepes (pH 7.9), 4 mM $MgCl_2$] overnight (Slide-A-Lyzer MINI Dialysis Units, Thermo, 10000MWCO, No.69570). The resultant reaction was diluted to 10 nM TFIIH to detect their ATPase activities by adding 200 nM AdMLP, 1mM TCEP and 1 μM [^{32}P]- γ-ATP as previously reported.[1]

Protein expression and purification. The full length human XPB (wild type, C342A, C342S or C342T XPB) was PCR amplified using the following primers(Table S1): XPB-for: TATTCT AGA GCC ACC ATG GGC AAA AGA GAC CGA G, XPB-rev: TAT ATA CTC GAG TTT CCT AAA GCG CTT GAA GAG. Wild type and mutant his-XPB proteins were purified from baculovirus driven insect cells as previously described.[1] Wild type TFIIH(XPB with STAP tag) and mutant C342 TFIIH were over-expressed in HEK293T as previously described, then the cell lysates was incubated with IgG beads for 2hrs and digested with TEV protease overnight at 4 °C, and the resulting supernatant were incubated with Steptavidin beads, then washed with 2mM biotin to get the proteins.

Radioactivity based TFIIH and XPB ATPase assay. The DNA-dependent ATPase assay was performed as previously described.[1] Briefly, a 10-µl reaction mixture contained 20 mM Tris (pH 7.9), 4 mM $MgCl_2$, indicated concentration of ATP, 1 µCi [γ-^{32}P]ATP (3000 Ci/mmol), 100 µg/ml BSA, indicated concentration of AdMLP, indicated concentration of TFIIH or his-XPB and indicated concentrations of triptolide. The reactions were started by either addition of TFIIH/his-XPB or ATP and incubated at 37 ^{0}C for indicated time. The reactions were stopped by addition of 2 µl of 0.5 M EDTA and dilution up to 100 µl with TE buffer. An aliquot of 1 µl reaction mixture was spotted on PEI-cellulose and the chromatogram was developed with 0.5 M LiCl and 1 M HCOOH. The percent of ATP hydrolysis was quantified using a PhosphorImager.

Knock-in C342T XPB in HEK293T cell line.[2-4] The overall workflow is outlined in **Figure S13**. A gRNA cloning vector and Cas9-D10A were purchased from Addgene (**Plasmid 41824: gRNA_Cloning Vector, Plasmid 41816: hCas9_D10A**). pCMV-hyPBase and pMCS-AAT-

PB:*PGKpuroΔtk* were from Wellcome Trust Sanger Institute. In-fusion HD cloning kit (Cat No. 639648) was from Agilent. Primers (Supplementary Table S2) were synthesized by IDT. The gRNA-XPB plasmid was constructed as previously described.[3] Briefly, the XPB-gRNA-for and XPB-gRNA-rev primers were annealed and extended by AccuPrime *Pfx* DNA polymerase (Invitrogen), then the 100bp fragment was cloned into Afl II linearized gRNA cloning vector using In-fusion HD cloning kit. XPB right arm fragments was obtained by XPB-R-F and XPB-R-R primers and cloned into pGEM-T vector, resulting the pGEM-XPB-R. The pGEM-XPB-R was mutated by XPB-mutant-for and XPB-mutant-rev primers to get pGEM-XPB-R-Mutant in which Cys342(TGC) was mutated to Thr342(ACC). Puromycin resistance gene, XPB left arm and right mutant arm fragments were obtained separately using Puro-for, Puro-rev, XPB-L-F, XPB-L-R, XPB-R-F and XPB-R-R primers from pMCS-AAT-PB:*PGKpuroΔtk*, genomic DNA and pGEM-XPB-R-Mutant, and cloned into EcoR V linearized pBluscript SK plasmid using In-fusion HD cloning kit to get donor-XPB plasmid.

100000 HEK293T cells in 24-wells plate were transfected with 1 μg Cas9-D10A plasmid, 1μg gRNA-XPB and 1 μg donor-XPB plasmid using lipofectamine 2000 as per manufacturer's instructions. Three days later, the cells were screened with 0.5 μg/ml puromycin. 7-10 days later, single clone was picked and cultured for another one or two weeks. Then total DNA was extracted and the clones were screened using PCR. Primers Endo-XPB-for2 and Endo-XPB-rev were used to confirm that one XPB allele was disrupted, and primers HR-Puro-for and Endo-XPB-rev were used to show that the puromycin gene was inserted in the correct position in the HEK293T genome. Disruption of one XPB allele in clone T7 was further confirmed by southern blot (**Figure S9a**).

For transposon excision, cell line T7 was transfected with pCMV-hyPBase and cultured for 4 days. Cells were then seeded at one cell per well in 96-wells plate. After one week, the clones were split into another 96-wells plate, and screened with 0.5μg/ml puromycin. Puromycin sensitive clone T71 was cultured sequentially and the total DNA was extracted. Primers HR-Puro-for and Endo-XPB-rev were used to show that the puromycin gene was removed from the HEK293T genome. Primers Endo-XPB-for2 and Endo-XPB-rev were used to confirm that one XPB copy was wild type and one XPB copy was C342T-XPB knock-in. The resultant PCR products were digested with AflII. Wild type XPB(1266bp) can't be digested by Afl II, but the Knock-in C342T-XPB(1266bp) can be digested into 260bp and 1006bp fragment (**Figure S9b)**.

100000 T71 cells per well of 24-well plate were transfected with 1 μg Cas9 plasmid, 1μg gRNA-XPB and 1 μg donor-XPB plasmid using lipofectamine 2000 as per manufacturer's instructions. Three days later, the cells were screened with 0.5 μg/ml puromycin. 7-10 days later, single clone was picked and cultured for another one or two weeks. Then total DNA was extracted and the clones were screened using PCR. Primers HR-Puro-for and Endo-XPB-rev were used to show that the puromycin gene was inserted in the correct position in the HEK293T genome. Primers Endo-XPB-for2 and Endo-XPB-rev were used to confirm that one XPB allele was disrupted, then the PCR products were digested with Afl II. If the PCR products can't be digested, it means that the cell clone only has one wild type XPB allele. Otherwise, the PCR products can be digested into 260bp and 1006bp fragment, it means that the cell clone only has the C342T-XPB knock-in cell line (**Figure S9C, T7115**). The PCR products were sequenced to confirm that the T7115 clone has the desired XPB configuration (**Figure S10**).

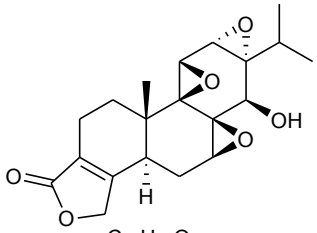
Table S1. XPB Mutant Primers

Primer Name	Sequence
XPB-C342A -for	GTC ATT GTT CTT CCC GCT GGT GCT GGA AAG TCC
XPB-C342A -rev	GGA CTT TCC AGC ACC AGC GGG AAG AAC AAT GAC
XPB-C342S -for	GTC ATT GTT CTT CCC TCC GGT GCT GGA AAG TCC
XPB-C342S -rev	GGA CTT TCC AGC ACC GGA GGG AAG AAC AAT GAC
XPB-C342T-for	GTC ATT GTT CTT CCC ACC GGT GCT GGA AAG TCC
XPB-C342T-rev	GGA CTT TCC AGC ACC GGT GGG AAG AAC AAT GAC
XPB-for:	TAT TCT AGA GCC ACC ATG GGC AAA AGA GAC CGA G
XPB-rev:	TAT ATA CTC GAG TTT CCT AAA GCG CTT GAA GAG

Table S2. XPB-C342T knock-in primers used in this work with a combination of CRISPR-Cas and *piggyBac* transposase technology

Primer name	Sequence
XPB-gRNA-for:	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC*G*AAACGGGCGTGCACGTTCG
XPB-gRNA-rev:	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC CGAACGTGCACGCCCGTTTC
XPB-mutant-for:	GGTCATTGTTCTTCCCACCGgtaagtggtaccag
XPB-mutnat-rev:	ctggtaccacttacCGGTGGGAAGAACAATGACC
XPB-L-F:	GTATCGATAAGCTTGAT *tggttctacccttacaacttc*
XPB-L-R:	GCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAGGTCAATGTTGATATCAGGG
XPB-R-F:	CGTACGTCACAATATGATTATCTTTCTAGGGTTAAGCCCACAGCTGTCCTCAG
XPB-R-R:	GGCTGCAGGAATTCGATccattaaacagagatccttcc
Puro-for:	AGTCTGCGTAAAATTGACGC
Puro-rev:	GATAATCATATTGTGACGTACG
HR-Puro-for:	CGTCAATTTTACGCATGATTATCTTTAAC
Endo-XPB-for-2:	ctttatcccggttgttgactgagca
Endo-XPB-rev:	tcggcaaaagaccactattattattacaca

Triptolide (**1**)

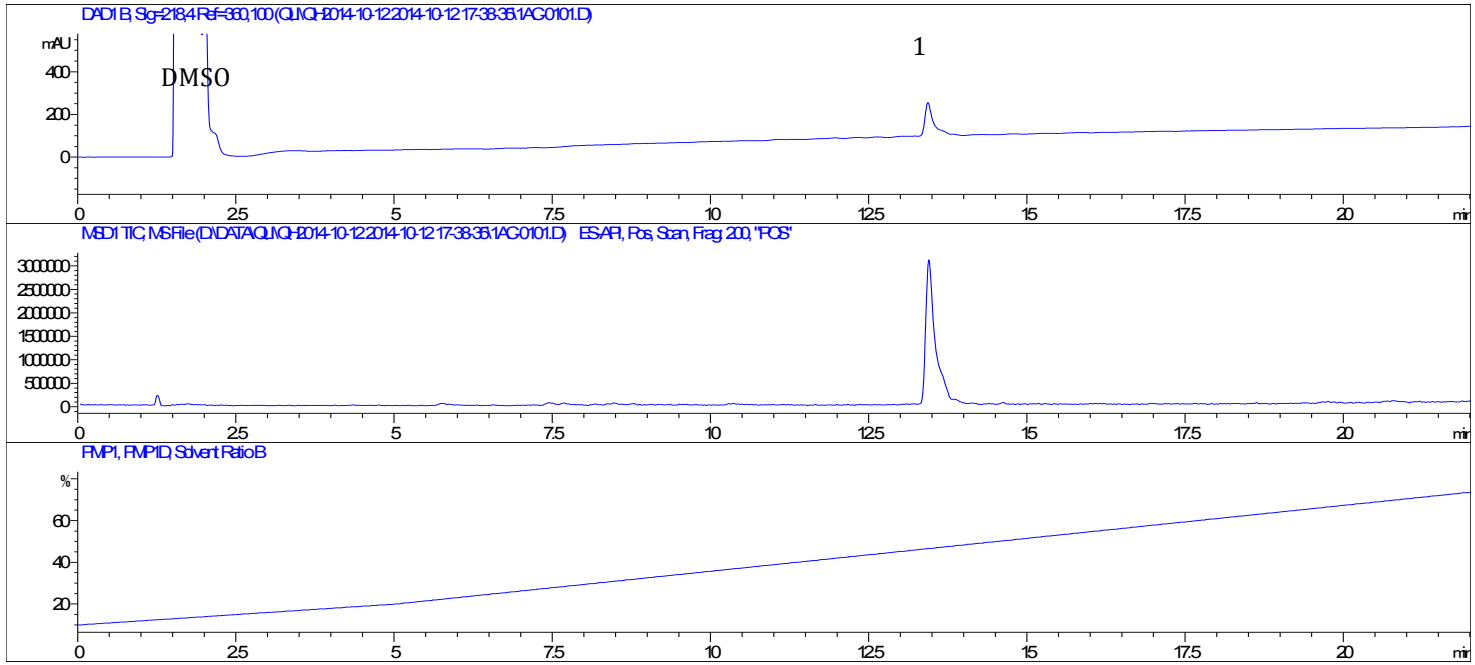


$C_{20}H_{24}O_6$
Exact Mass: 360.1573
Mol. Wt.: 360.401

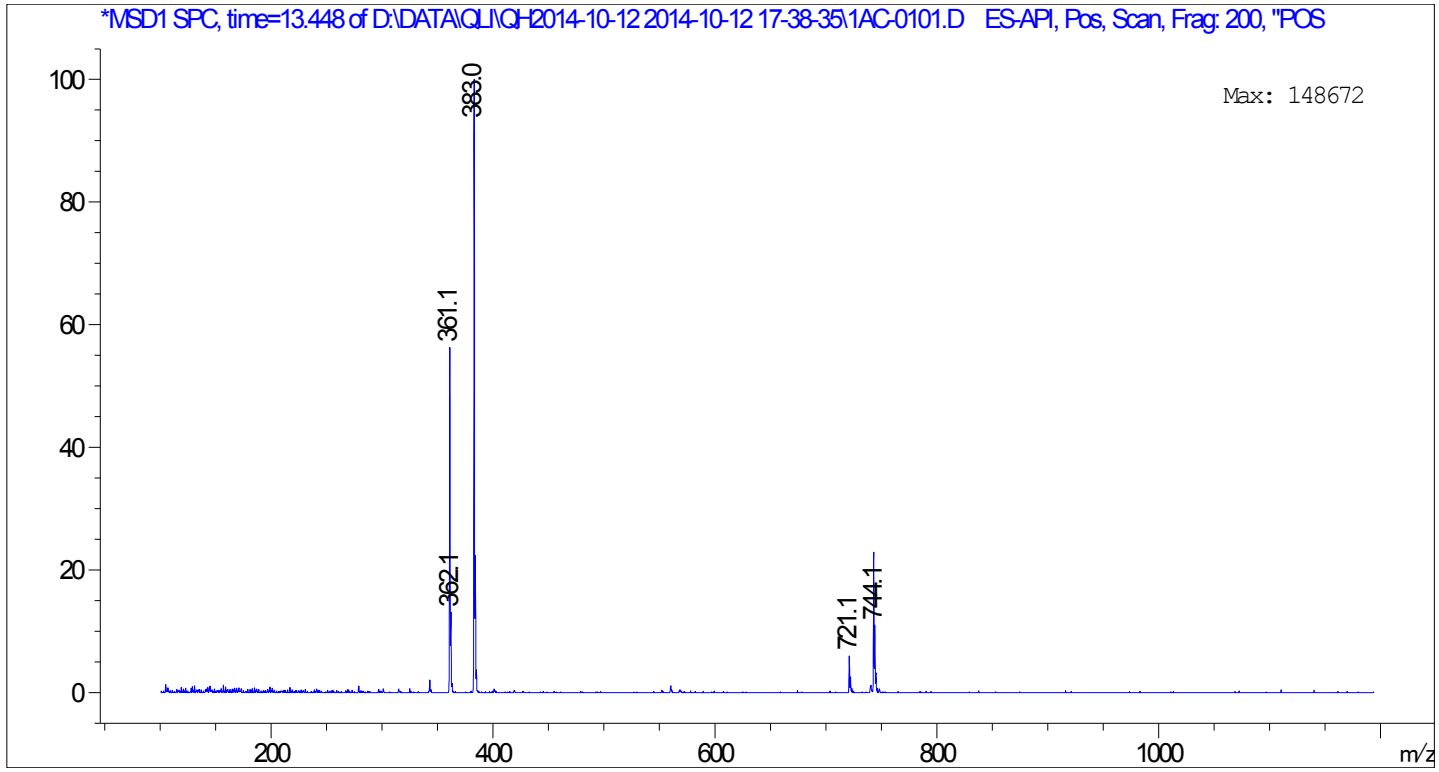
HPLC

TIC

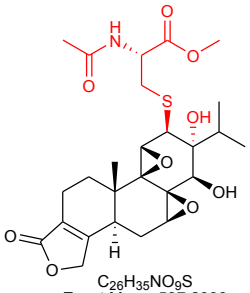
Solvent ratio



Major Peak (positive)



Analog **4**

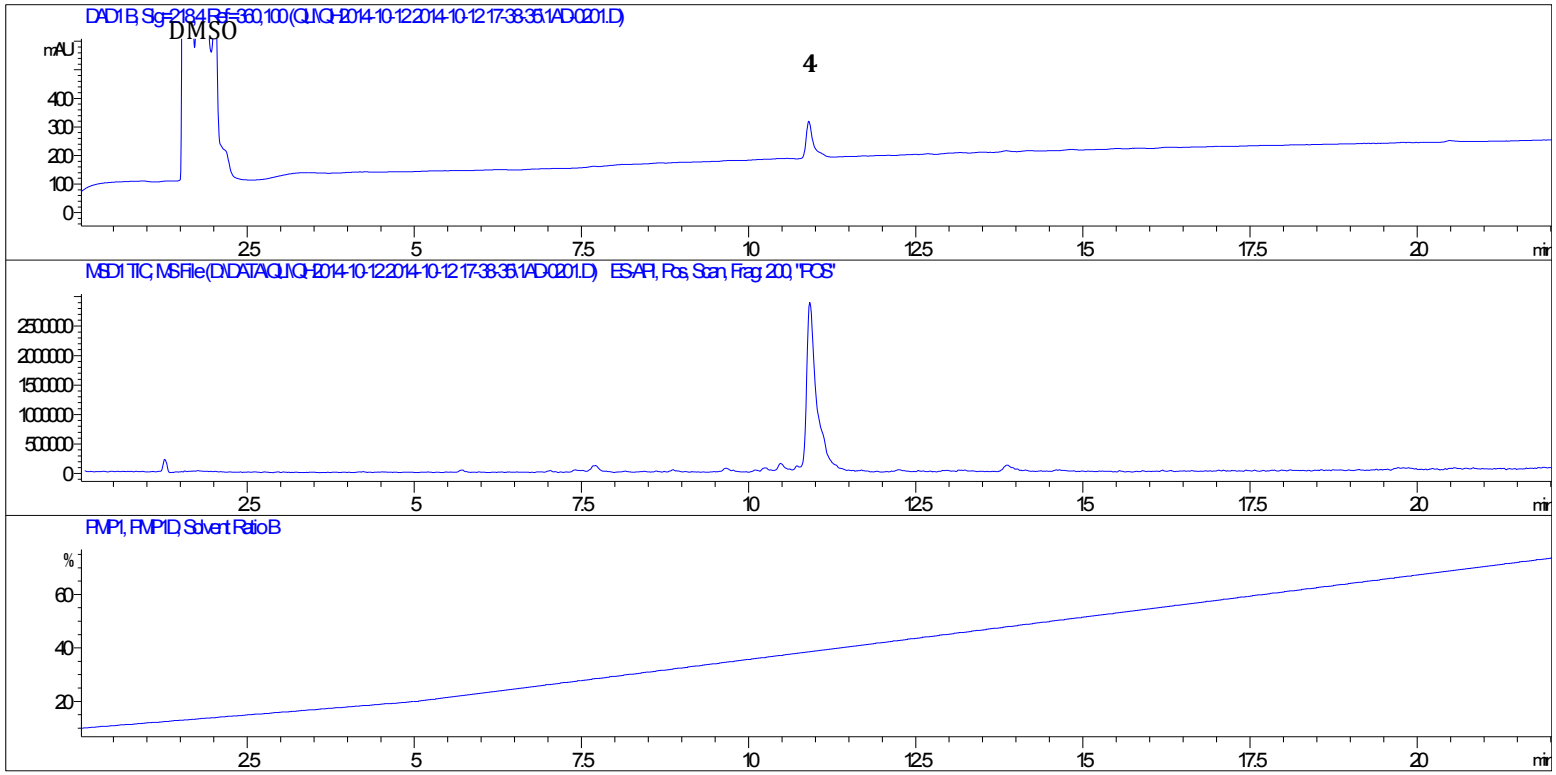


$C_{26}H_{35}NO_9S$
Exact Mass: 537.2033
Mol. Wt.: 537.6224

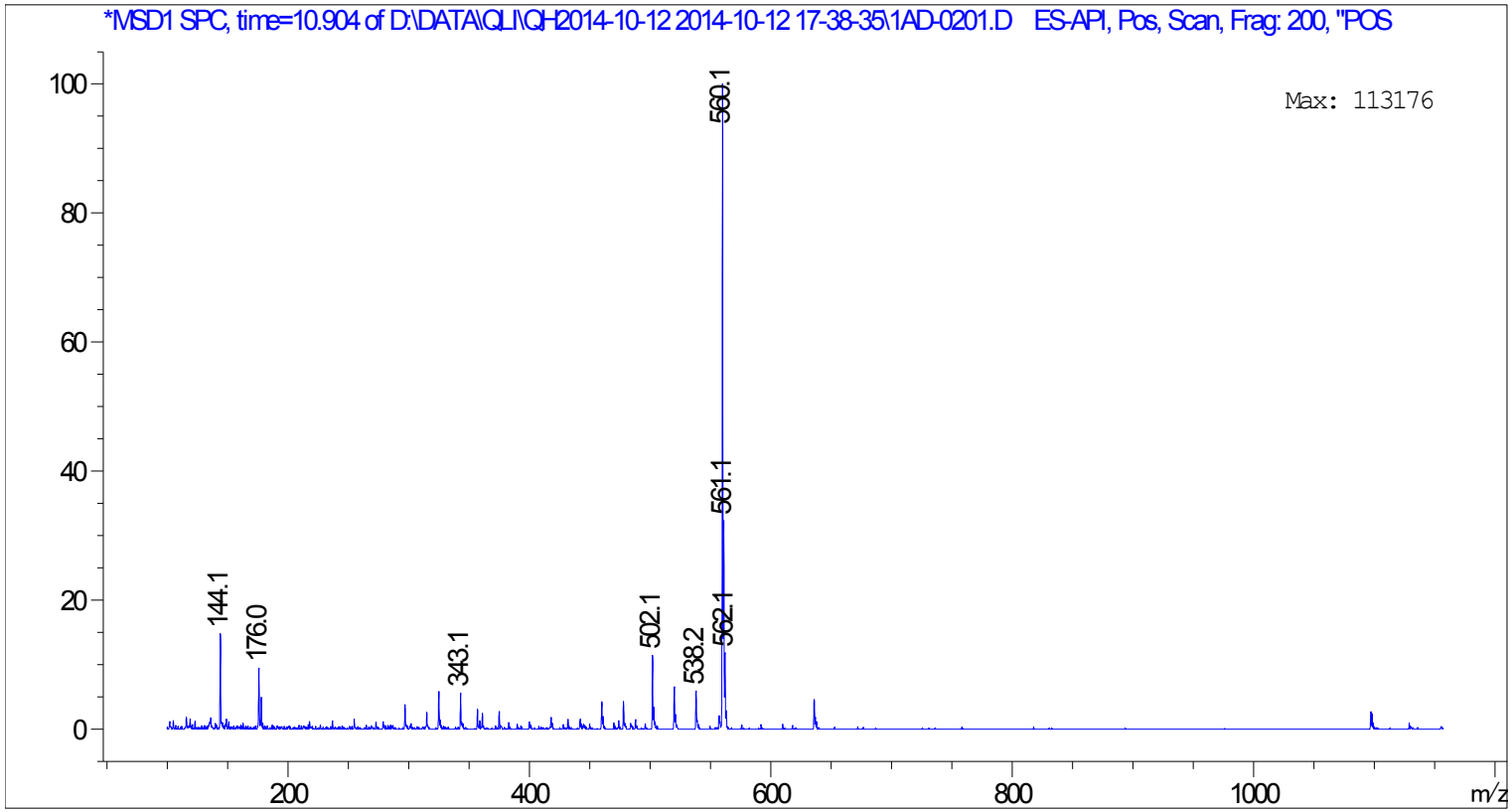
HPLC

TIC

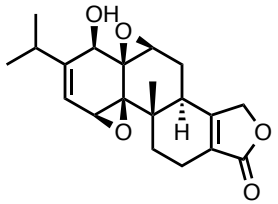
Solvent ratio



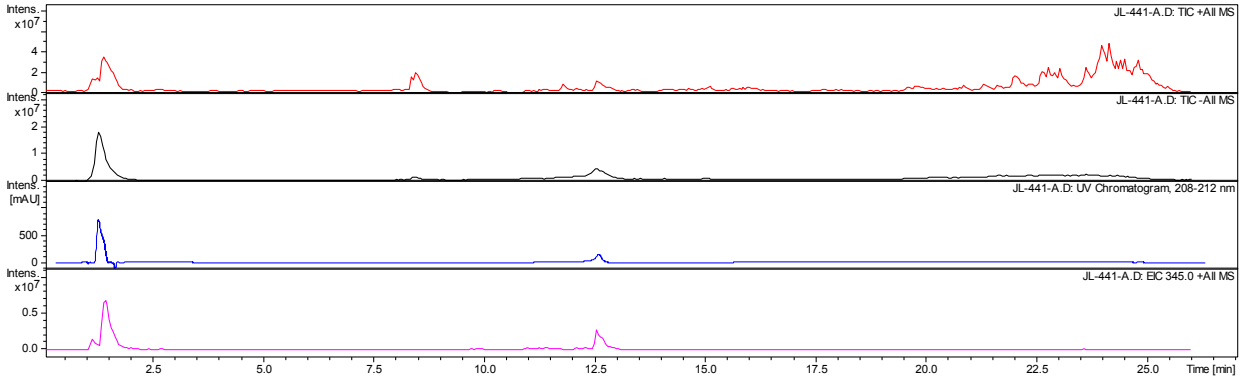
Major Peak (positive)



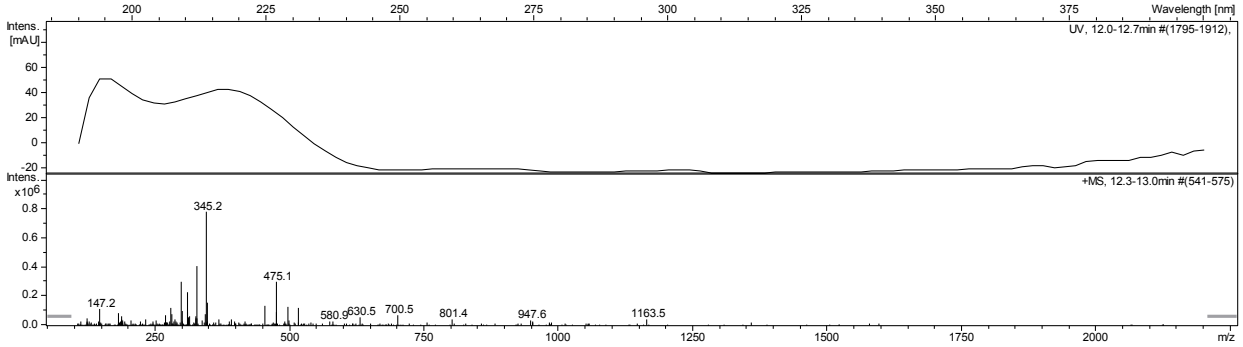
Analog **5**:



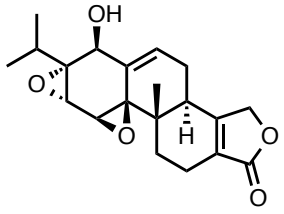
Chemical Formula: $C_{20}H_{24}O_5$
Exact Mass: 344.1624
Molecular Weight: 344.4070



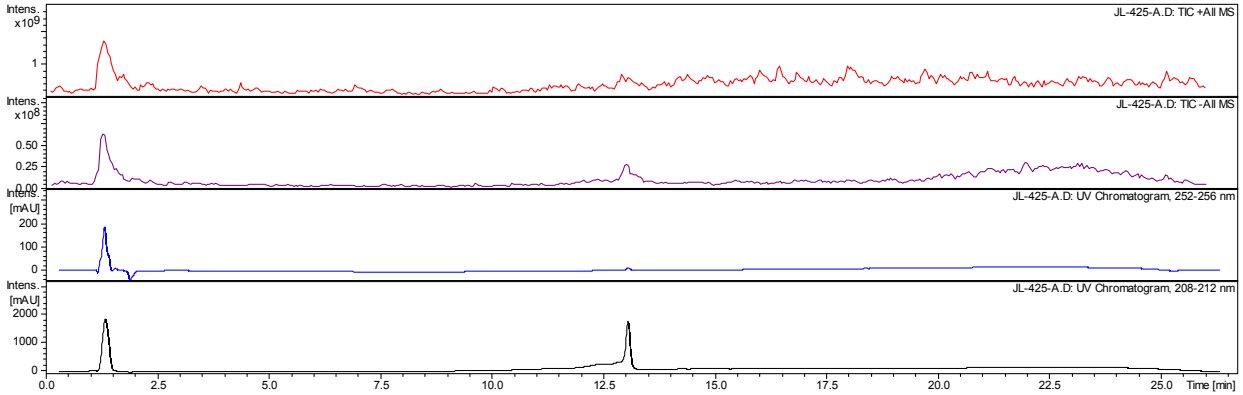
Major peaks (+ mode):



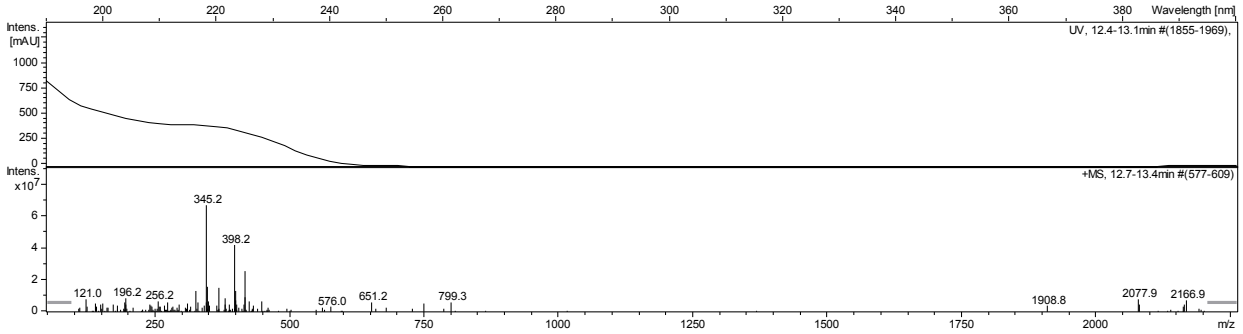
Analog **6**



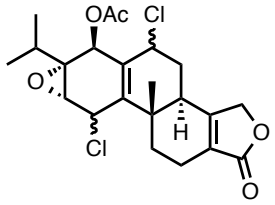
Chemical Formula: $C_{20}H_{24}O_5$
Exact Mass: 344.1624
Molecular Weight: 344.4016



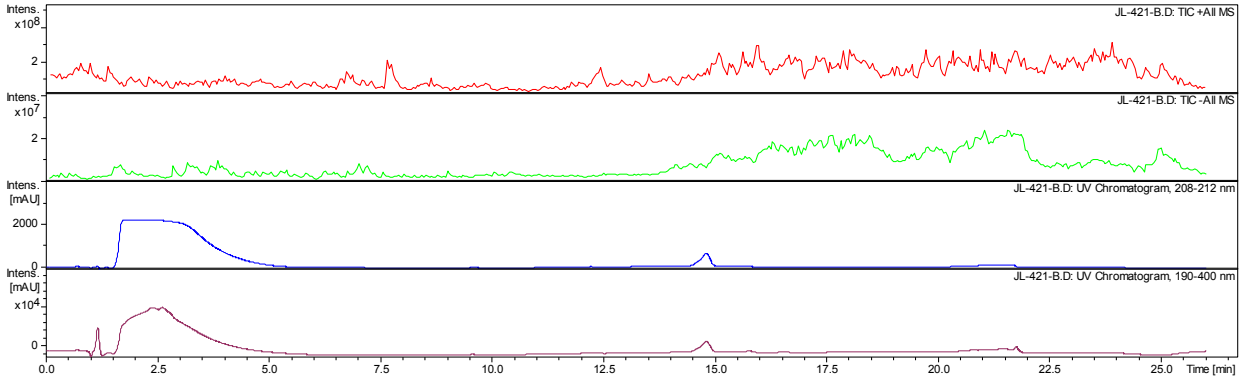
Major peaks (positive mode):



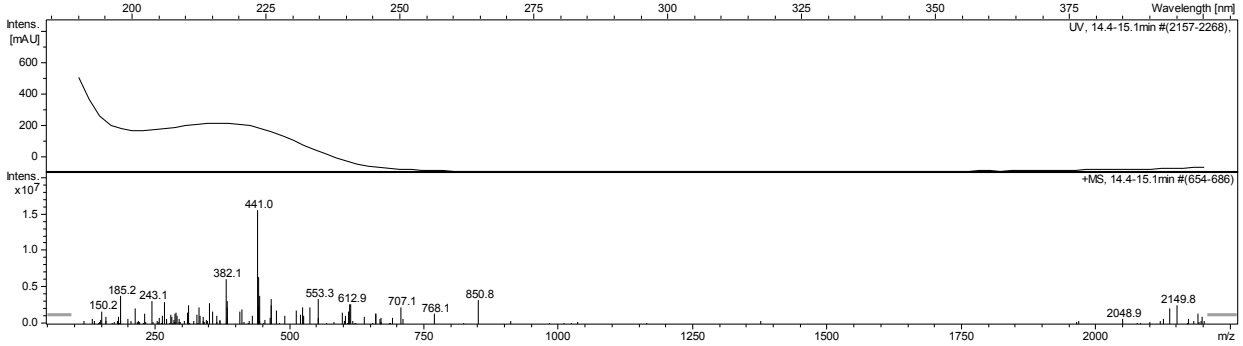
Analog **7**:



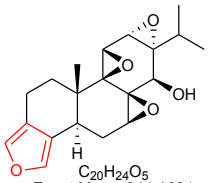
Chemical Formula: $C_{22}H_{26}Cl_2O_5$
Exact Mass: 440.1157
Molecular Weight: 441.3450



Major peaks (positive mode):



Analog **8**

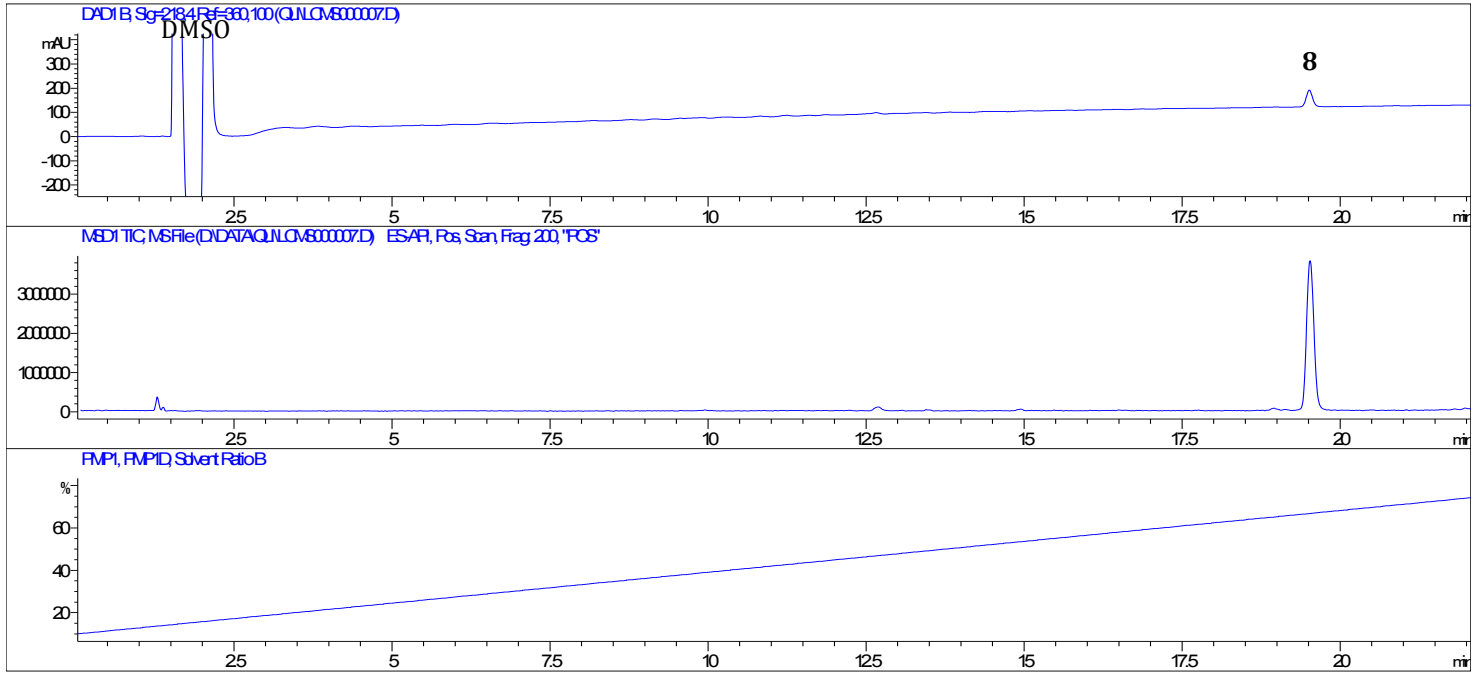


$C_{20}H_{24}O_5$
Exact Mass: 344.1624
Mol. Wt.: 344.4016

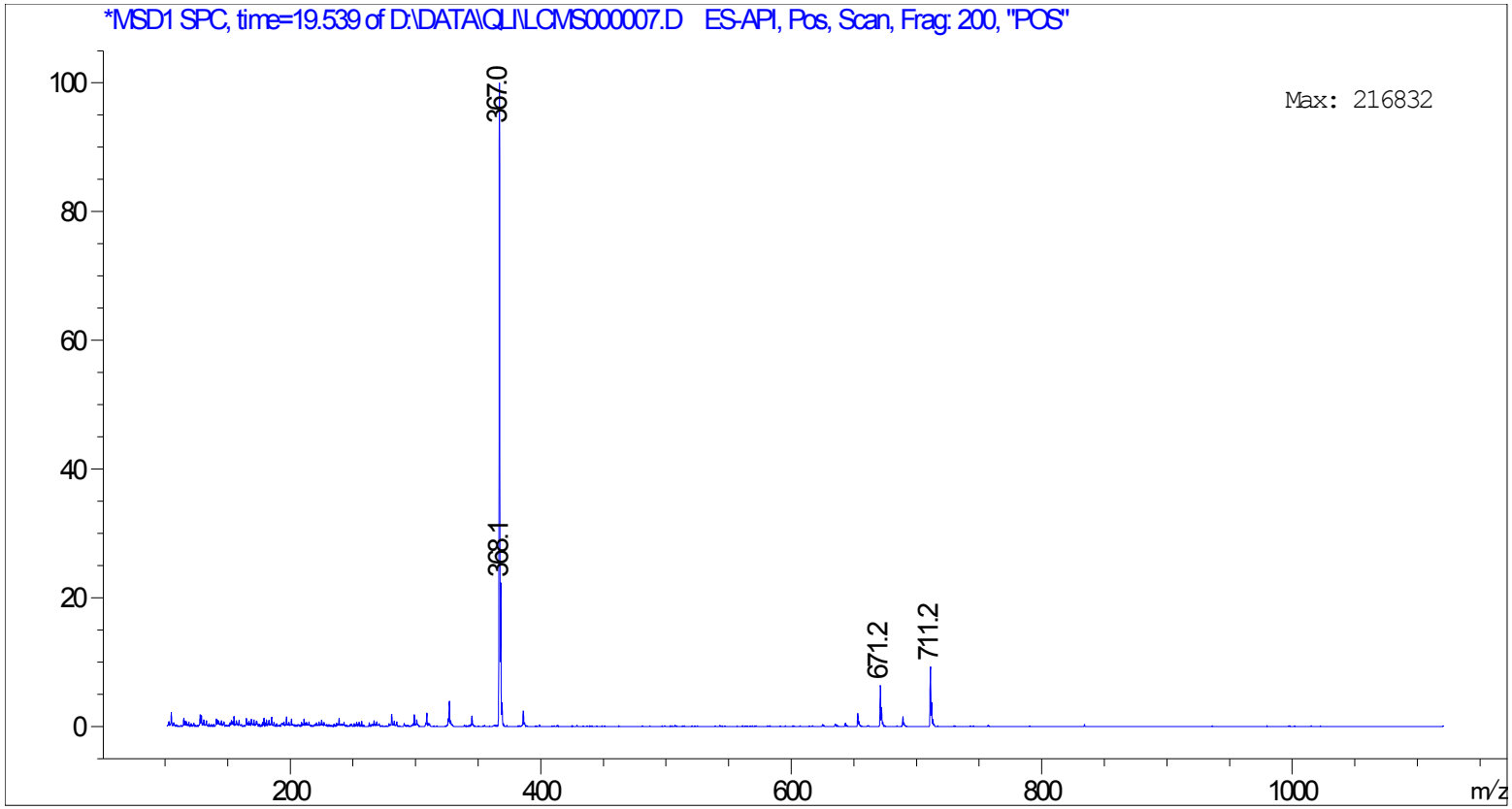
HPLC

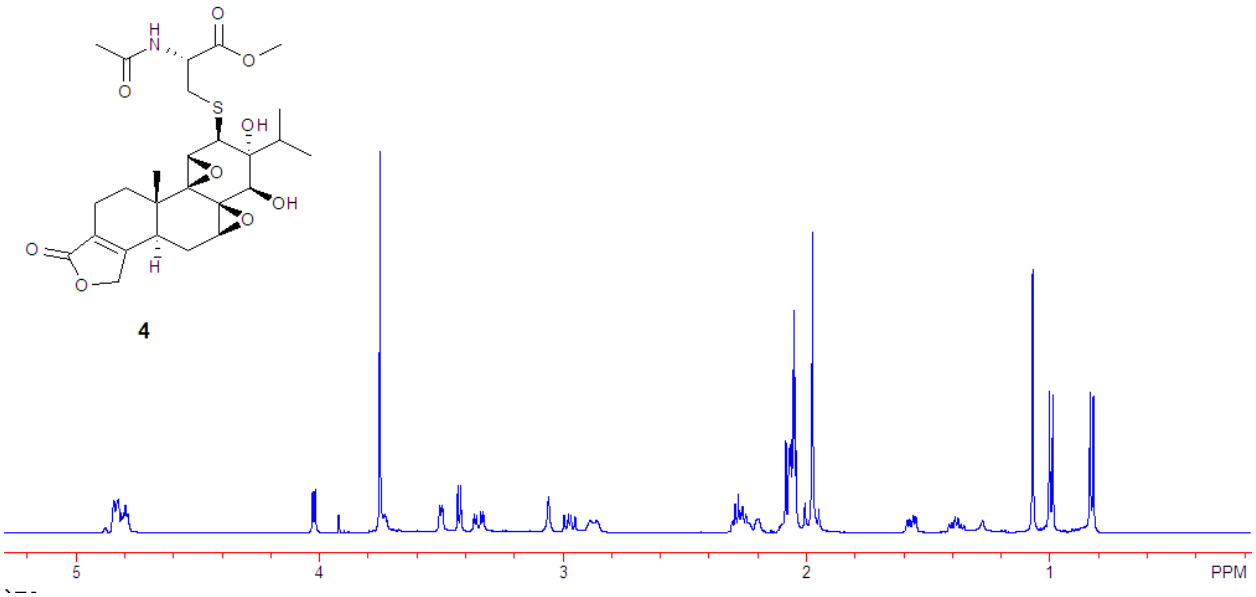
TIC

Solvent ratio

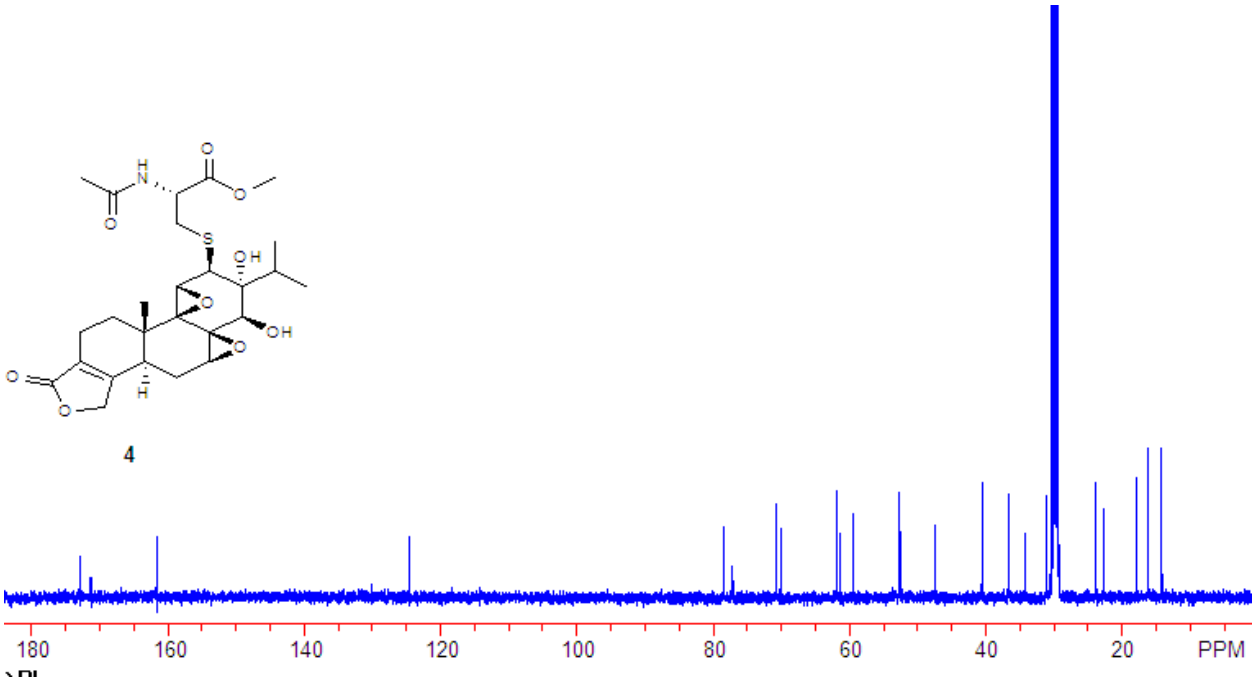


Major Peak (positive)

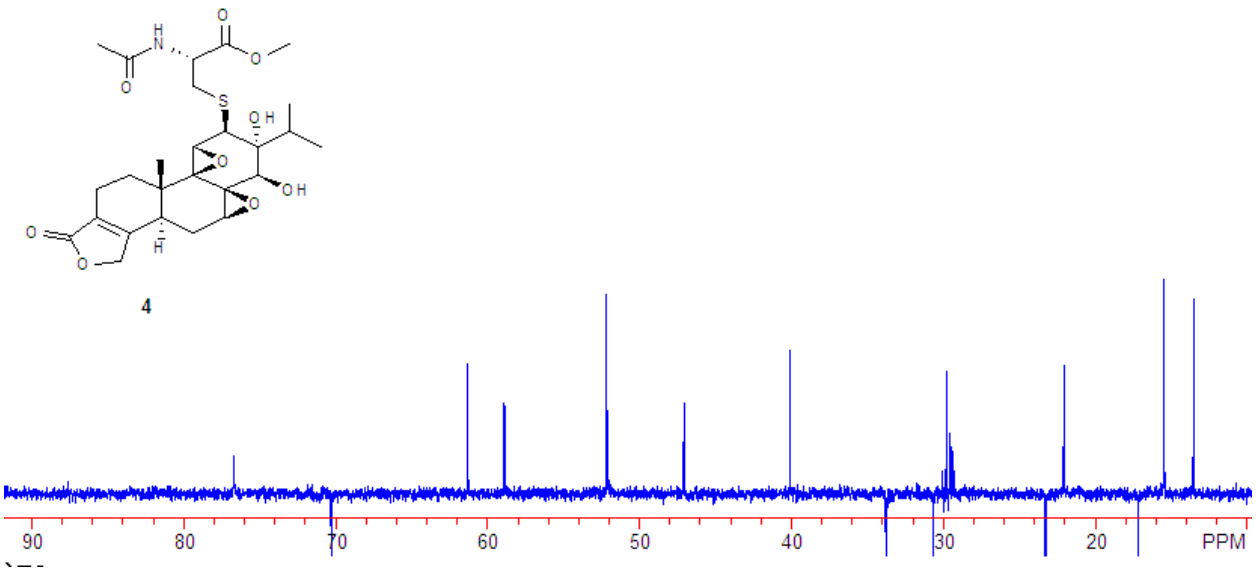




^{1}H(500 MHz) NMR of **4** in $((CD_3)_2CO$

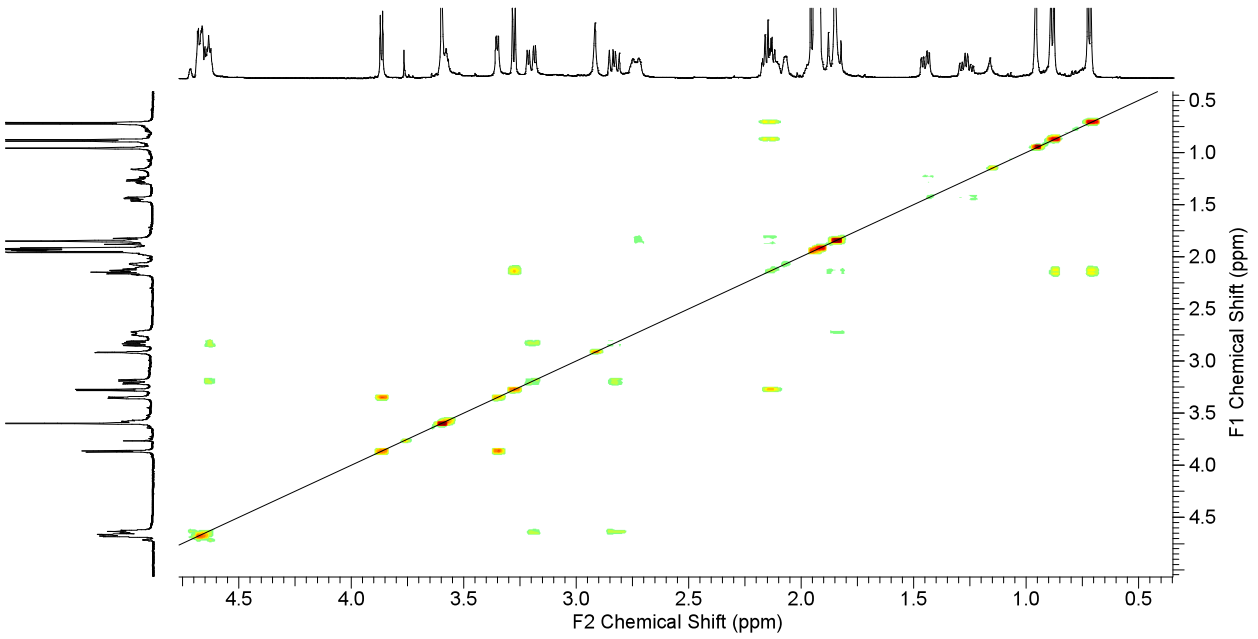


^{13}C(125 MHz) NMR of **4** in $((CD_3)_2CO$

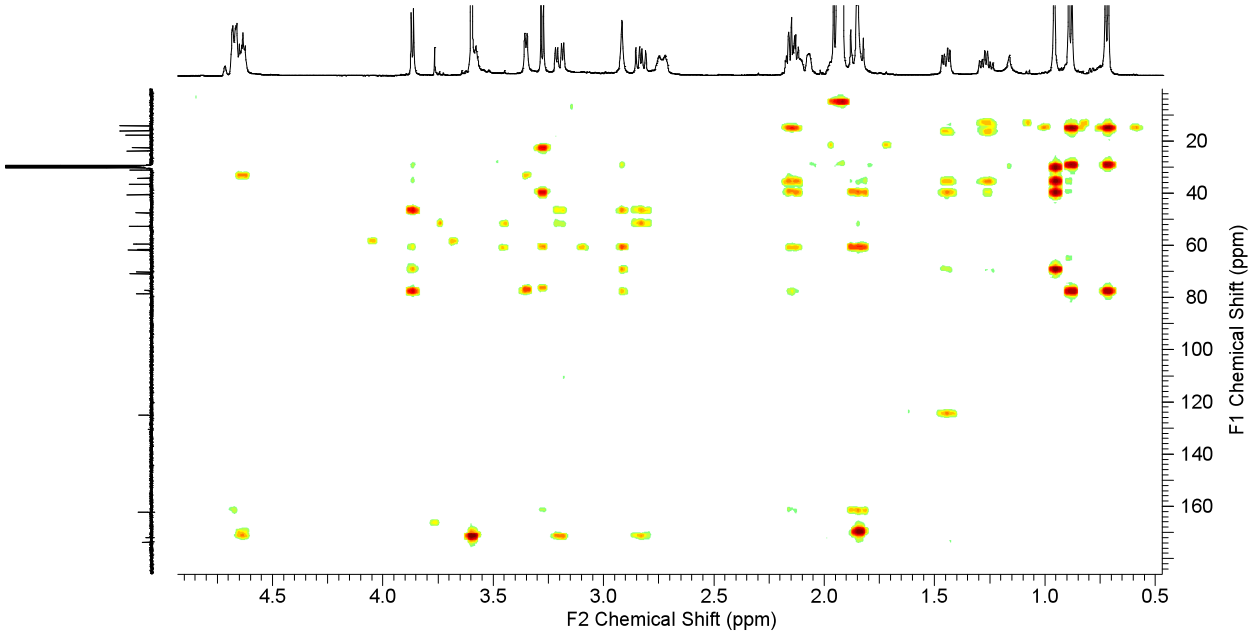


4

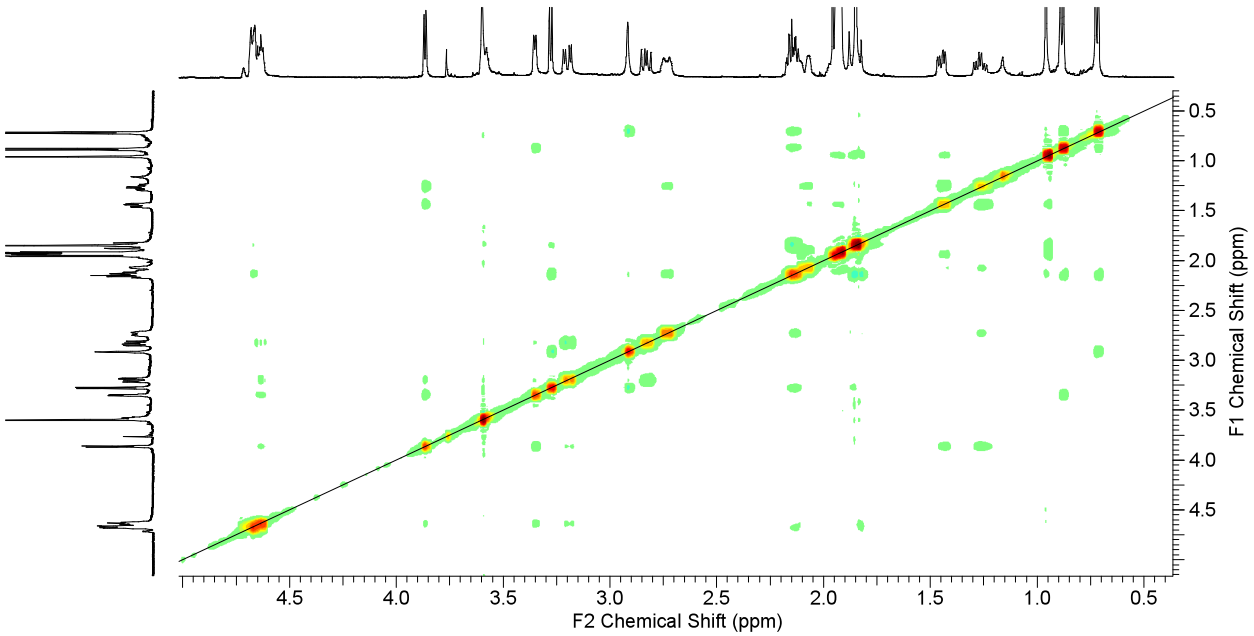
DEPT-135 NMR of **4** in $((CD_3)_2CO$



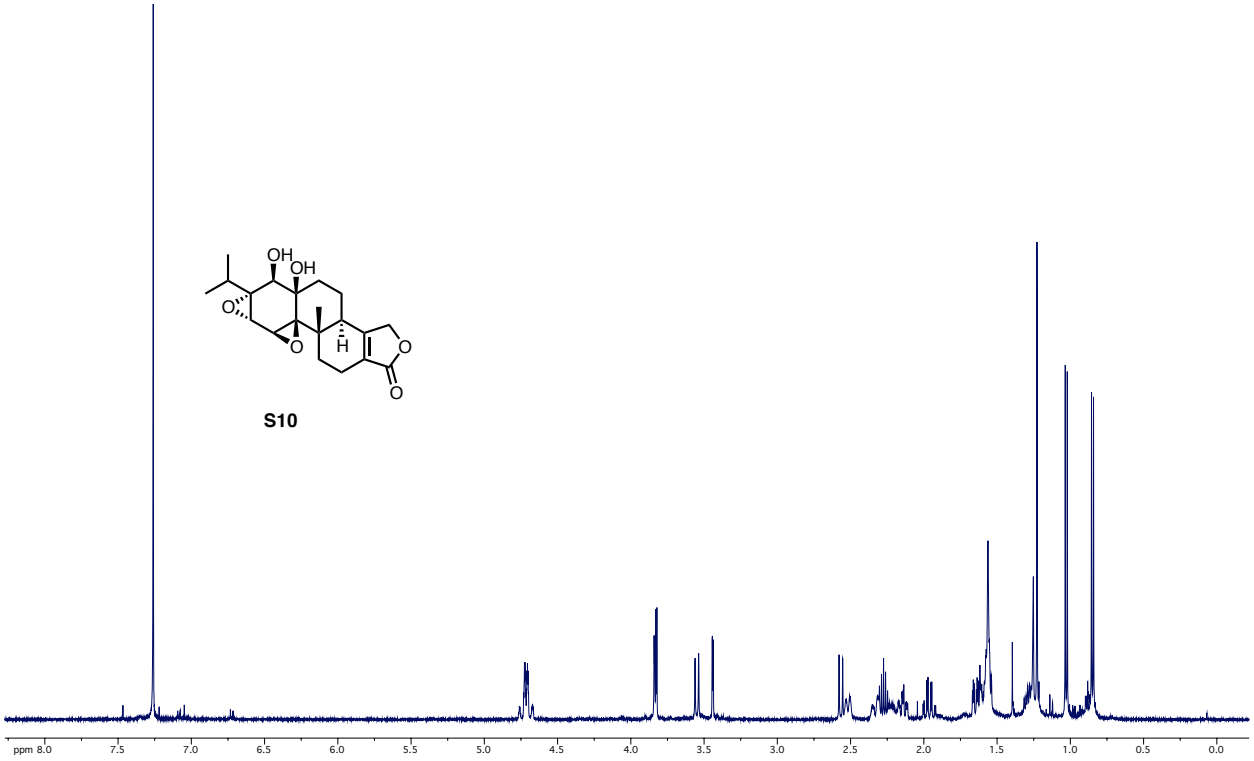
^{1}H-^{1}H Cosy (500 MHz) NMR of **4** in $((CD_3)_2CO$



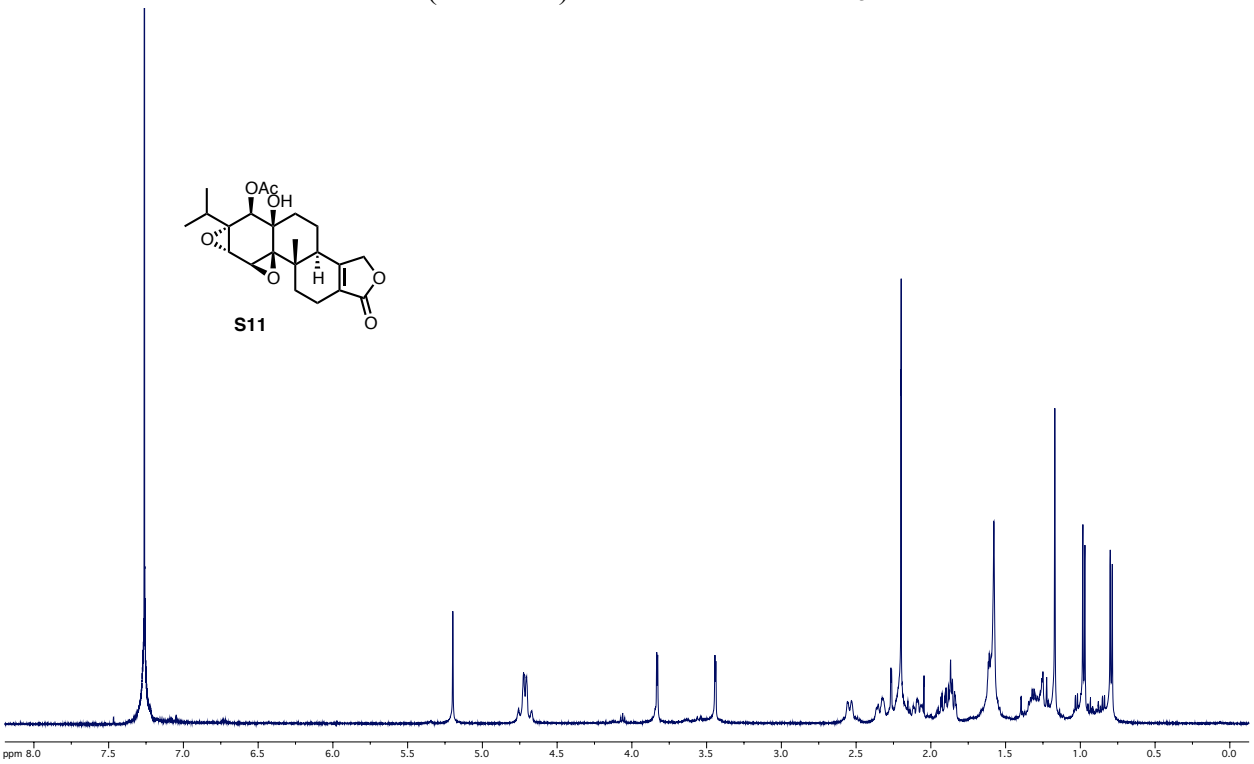
^{1}H-^{13}C HMBC NMR of **4** in $((CD_3)_2CO$



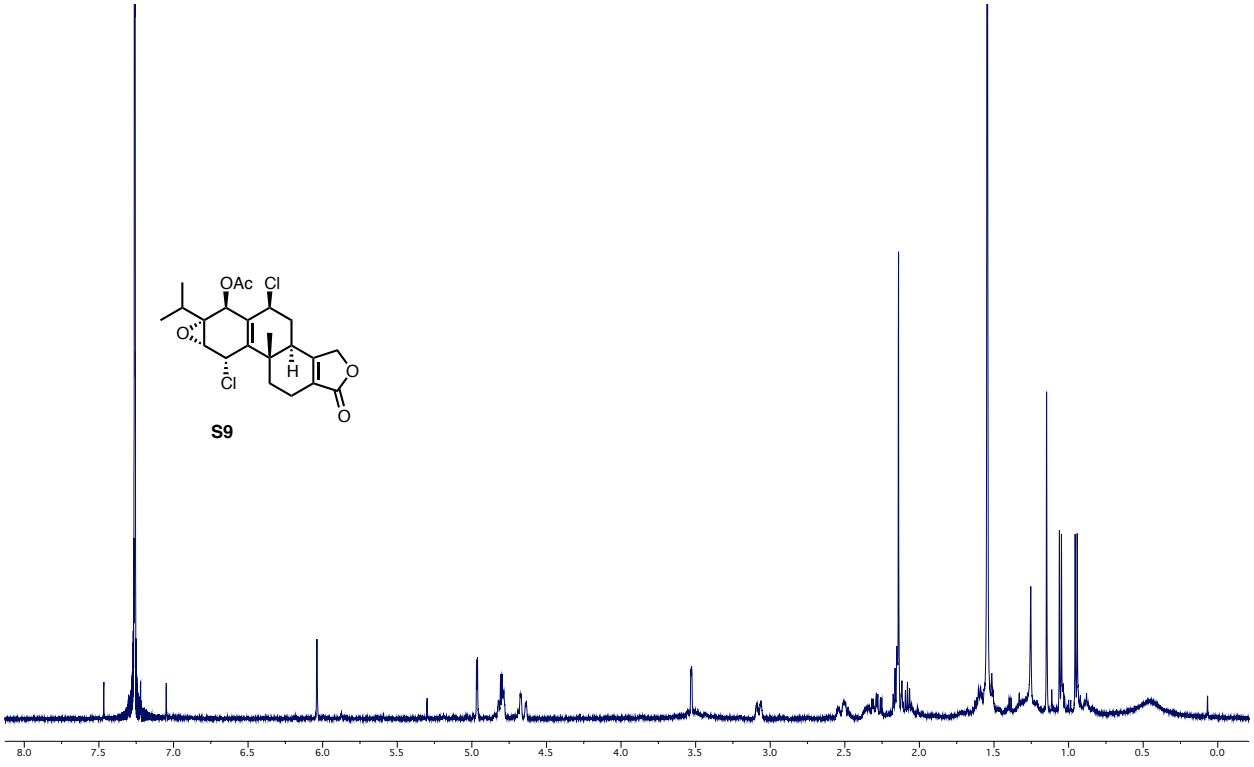
^{1}H-^{1}H NOESY(500 MHz) NMR of **4** in $((CD_3)_2CO$



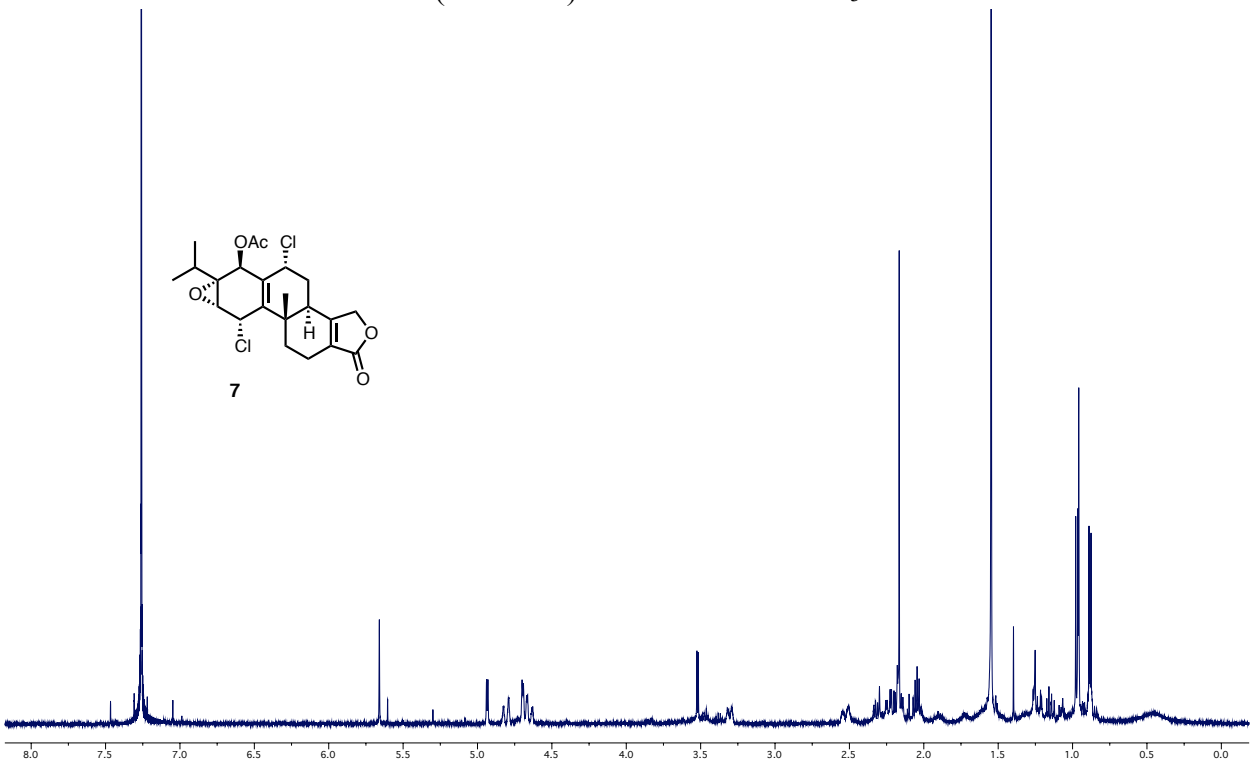
^{1}H (500 MHz) NMR of **S10** in $CDCl_3$



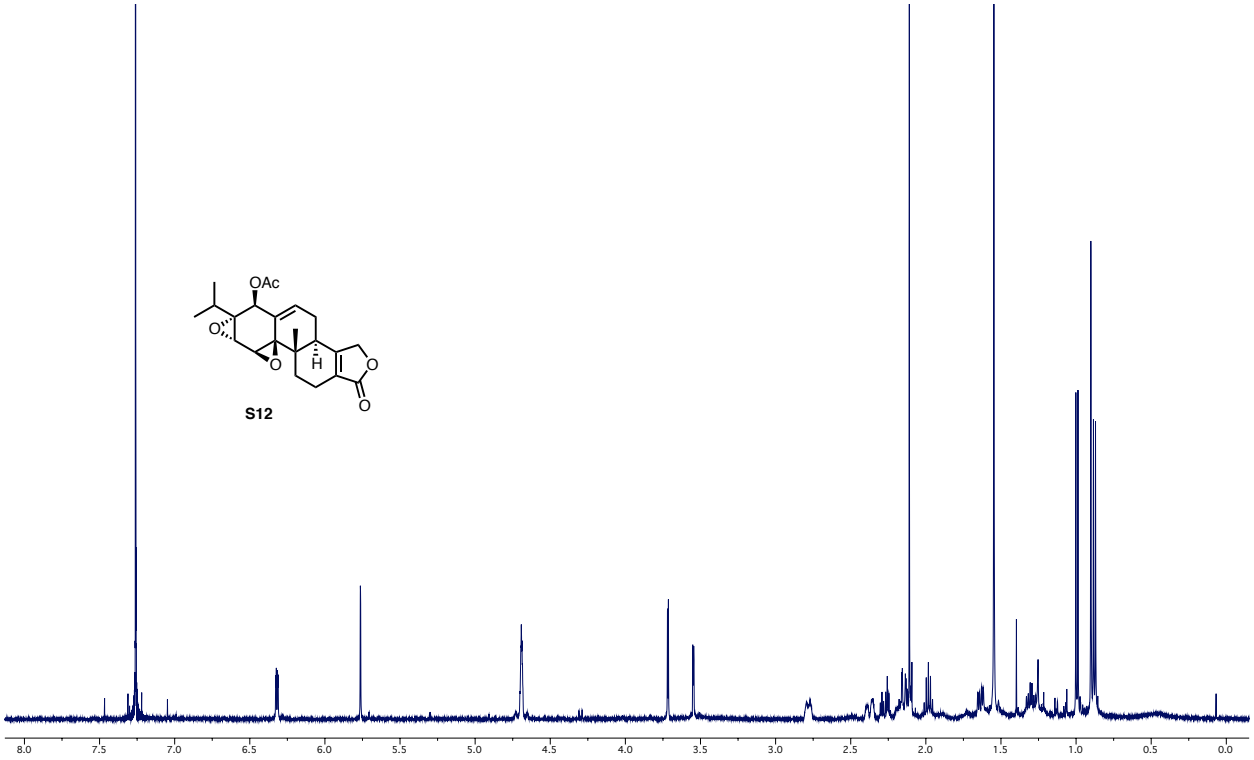
^{1}H (500 MHz) NMR of **S11** in $CDCl_3$



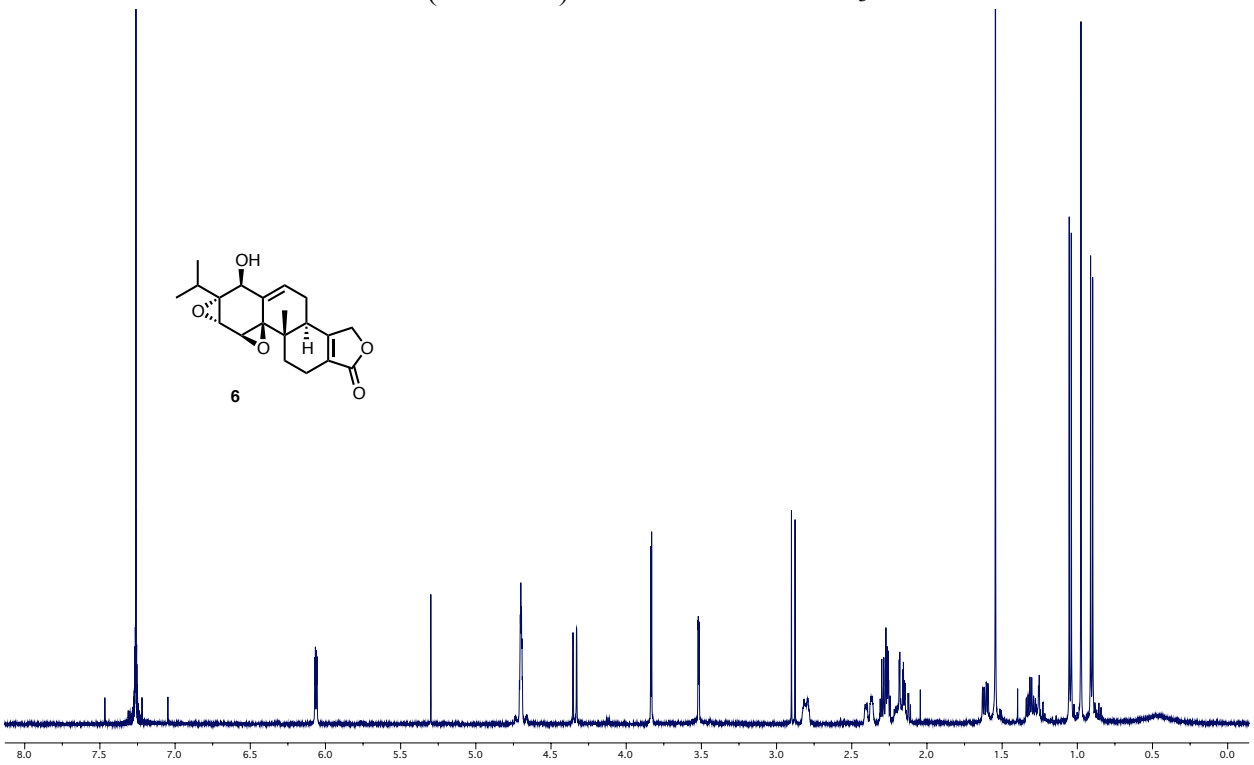
^{1}H (500 MHz) NMR of **S9** in $CDCl_3$



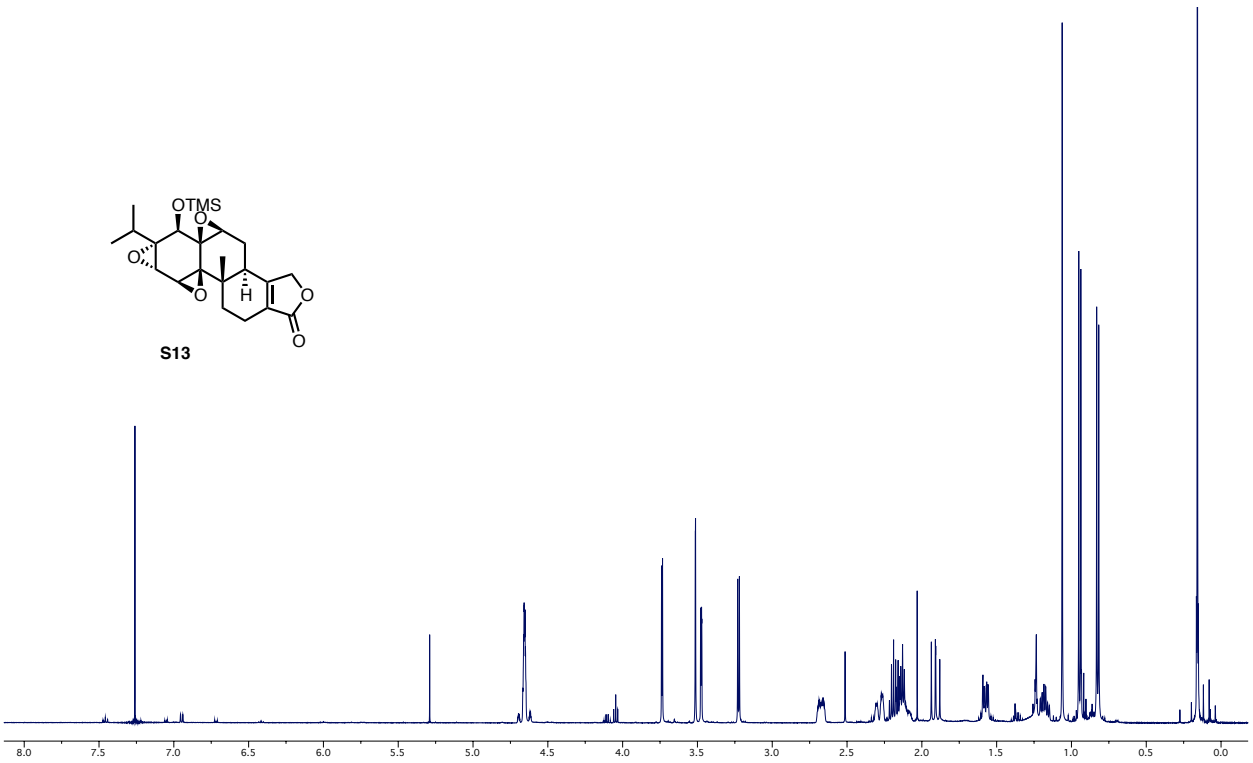
^{1}H (500 MHz) NMR of **7** in $CDCl_3$



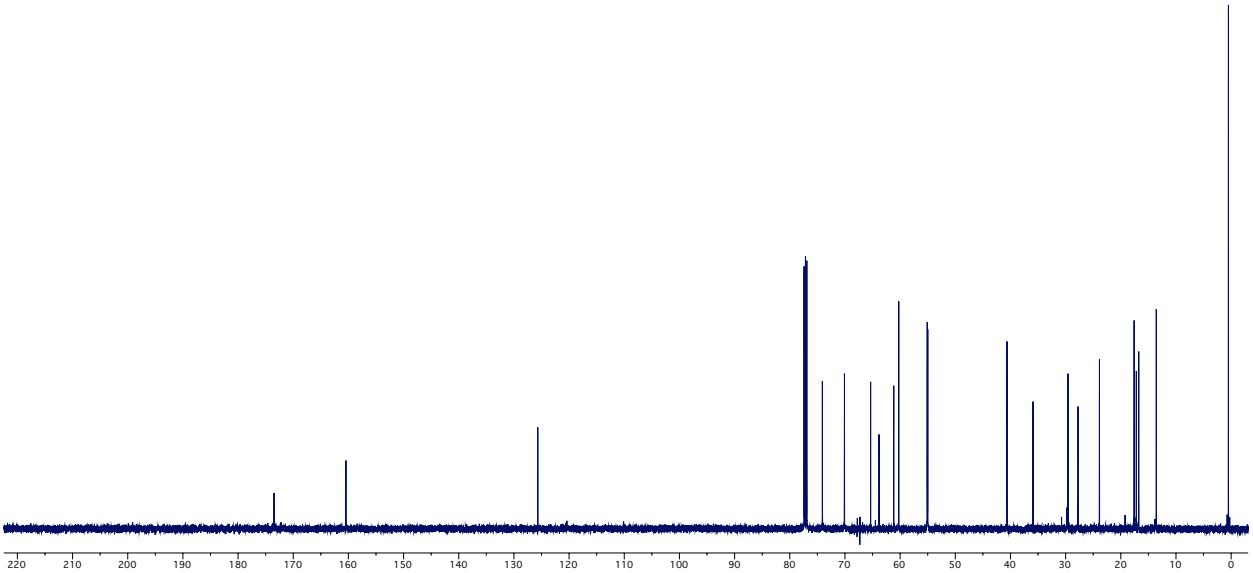
^{1}H (500 MHz) NMR of **S12** in $CDCl_3$



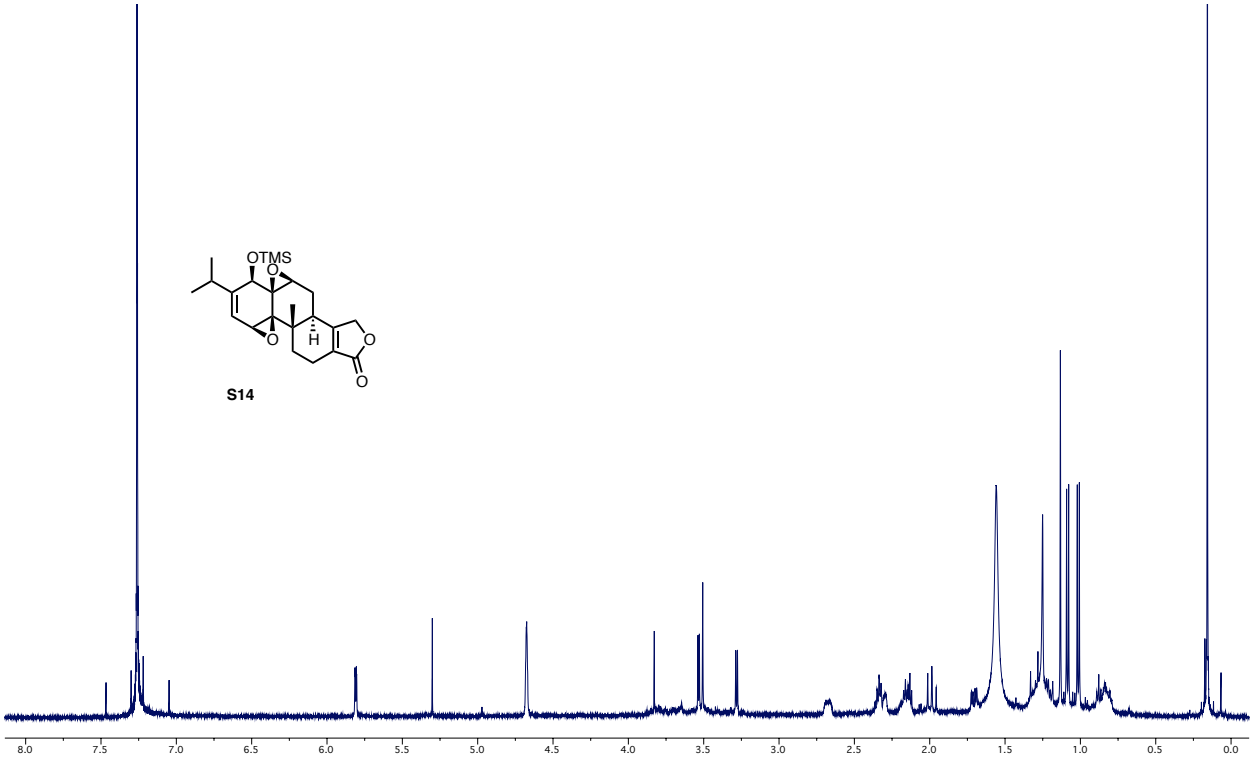
^{1}H (500 MHz) NMR of **6** in $CDCl_3$



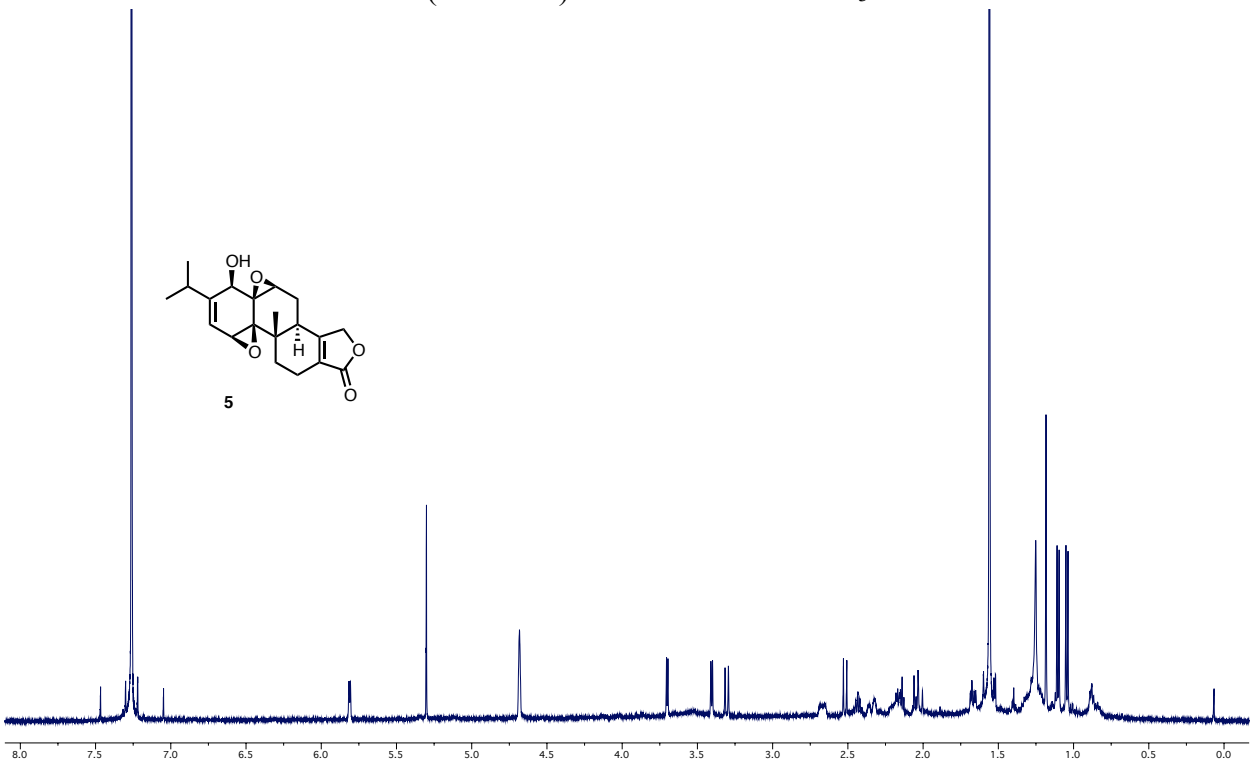
^{1}H (500 MHz) NMR of **S13** in $CDCl_3$



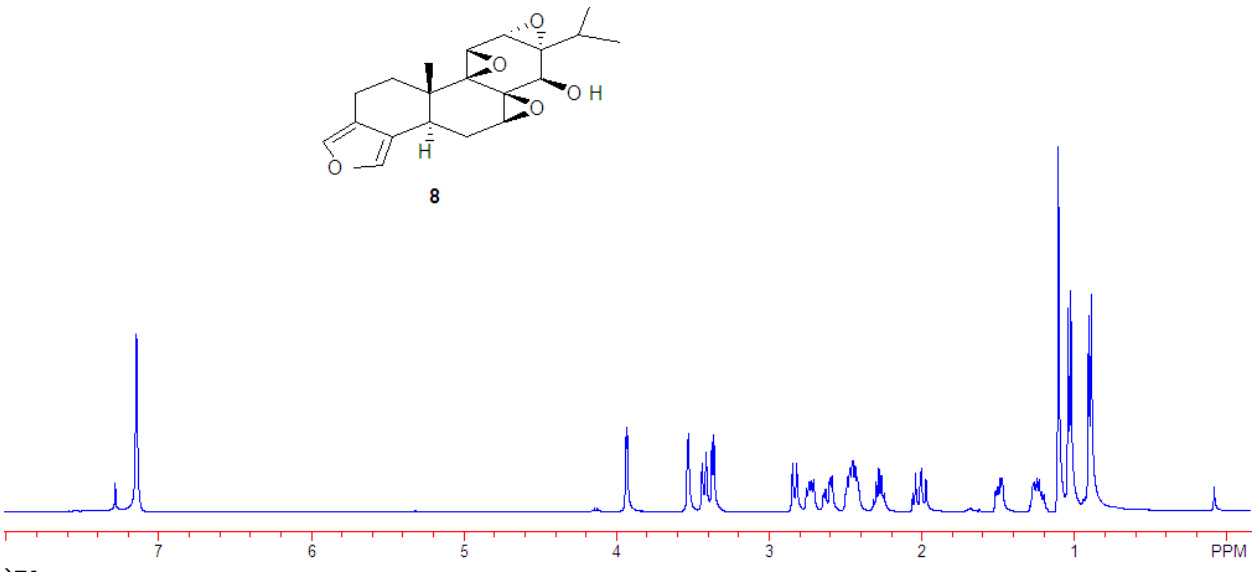
^{13}C (125 MHz) NMR of **S13** in $CDCl_3$



^{1}H (500 MHz) NMR of **S14** in $CDCl_3$

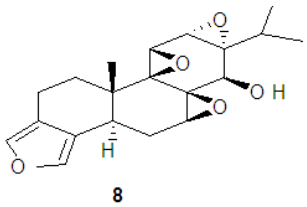


^{1}H (500 MHz) NMR of **5** in $CDCl_3$

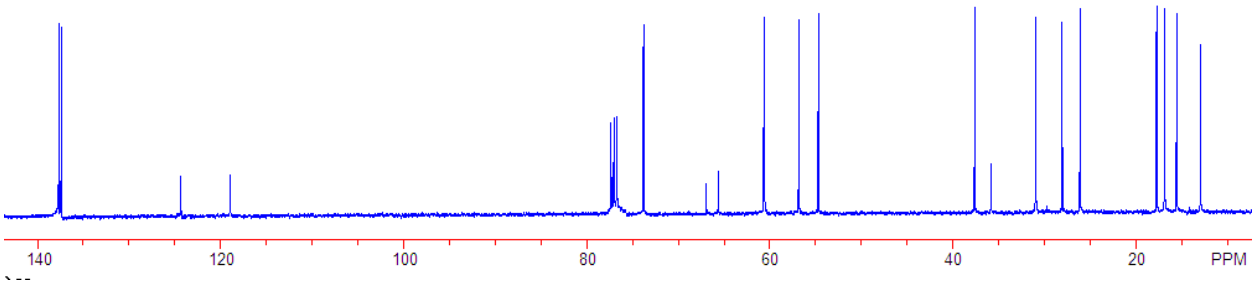


8

^{1}H(400 MHz) NMR of **8** in $CDCl_3$



8



^{13}C(100 MHz) NMR of **8** in $CDCl_3$

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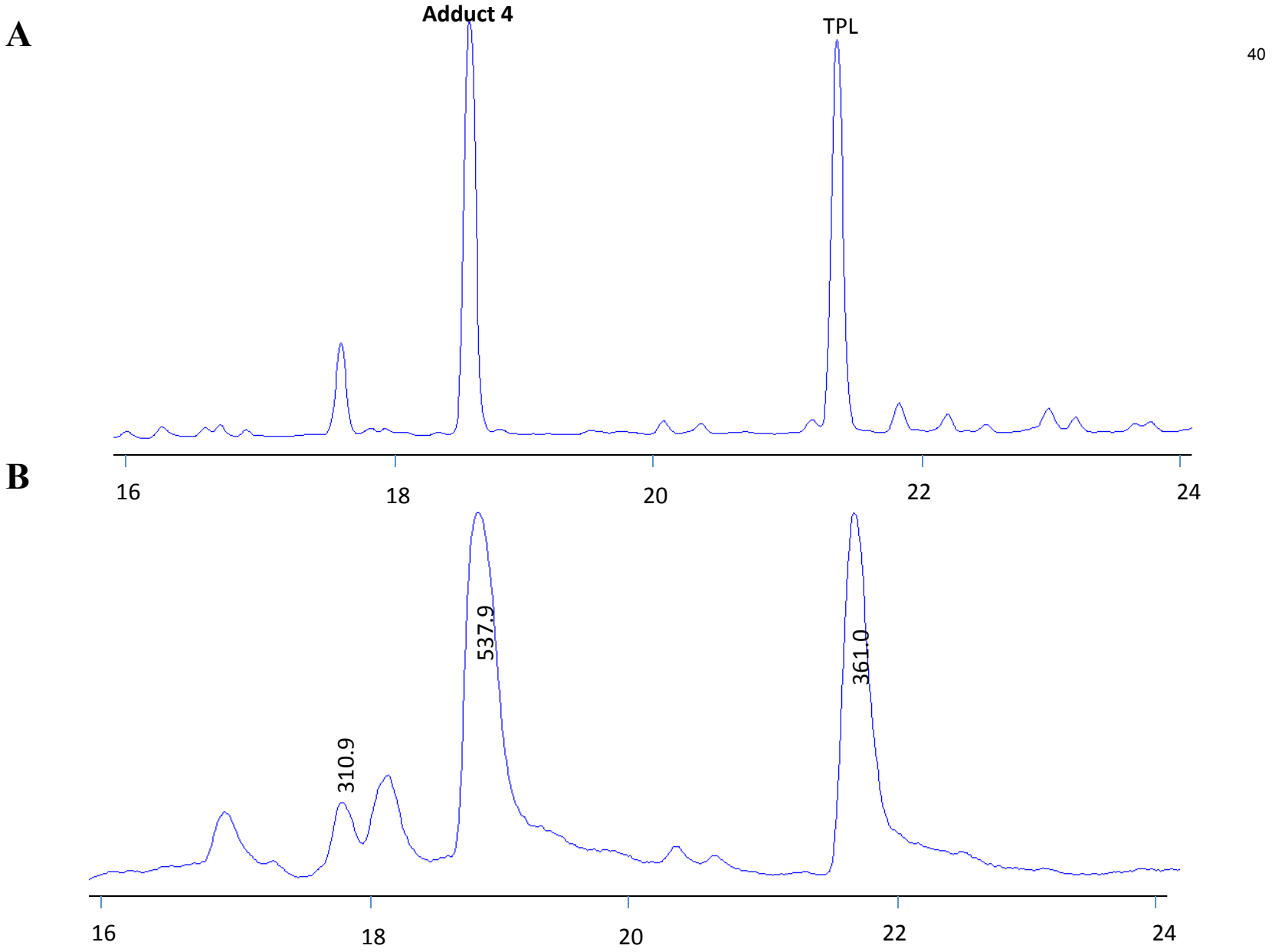

Fig. S1. HPLC and MS analysis of the products of reaction between TPL and N-acetyl-L-cysteine methyl ester. (**A**) HPLC analysis of the reaction products of TPL and N-acetyl-L-cysteine methyl ester **4**. (**B**) LC-MS (ESI, pos) analysis of the reaction products.

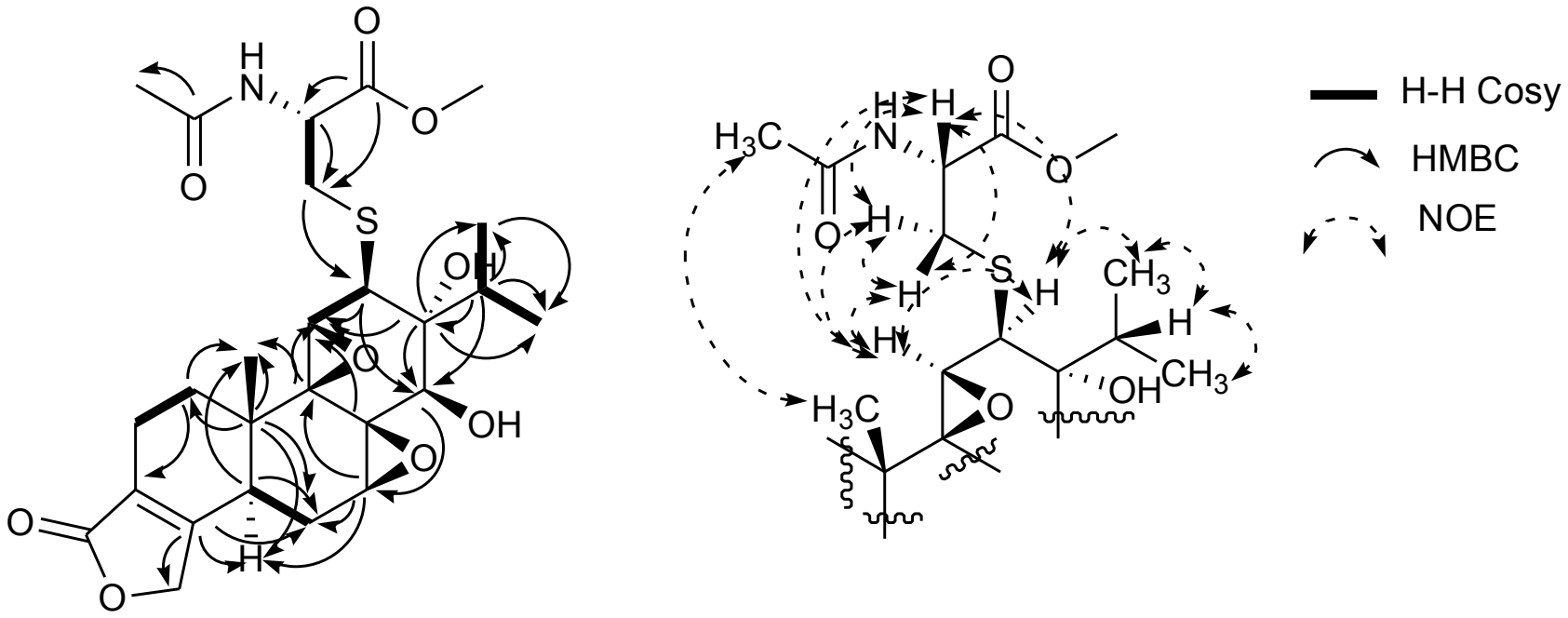

Fig. S2. The 2D-NMR analysis of the triptolide-cysteine adduct **4**.

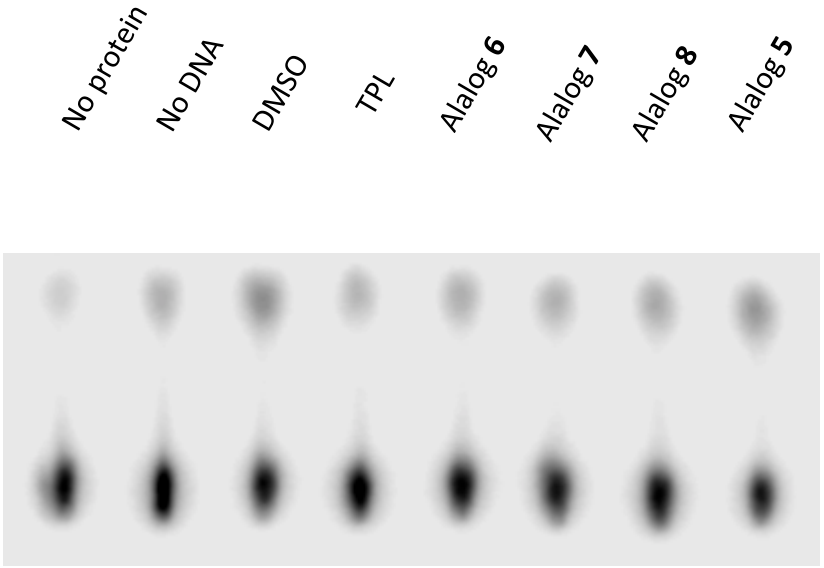

Fig. S3. Binding-dialysis-activity recovery assay. Different Triptolide analogs (100 μM) were incubated with TFIIH complex for 2 h. The reaction mixture were dialyzed to remove unbound small molecules and the remaining ATPase activities were determined using TLC assay.

Sequence Coverage: **82%**

Matched peptides shown in **Bold Red**

```
  1 MGKRDRADRD KKKSRKRHYE DEEDDEEDAP GNDPQEAVPS AAGKQVDESG
 51 TKVDEYGAKD YRLQMPLKDD HTSRPLWVAP DGHIFLEAFS PVYKYAQDFL
101 VAIAEPVCRP THVHEYKLTA YSLYAAVSVG LQTSDITEYL RKLSKTGVPD
151 GIMQFIKLCT VSYGKVKLVL KHNRYFVESC HPDVIQHLLQ DPVIRECRLR
201 NSEGEATELI TETFTSKSAI SKTAESSGGP STSRVTDPQG KSDIPMDLFD
251 FYEQMDKDEE EEEETQTVSF EVKQEMIEEL QKRCIHLEYP LLAEYDFRND
301 SVNPDINIDL KPTAVLRPYQ EKSLRKMFGN GRARSGVIVL PCGAGKSLVG
351 VTAACTVRKR CLVLGNSAVS VEQWKAQFKM WSTIDDSQIC RFTSDAKDKP
401 IGCSVAISTY SMLGHTTKRS WEAERVMEWL KTQEWGLMIL DEVHTIPAKM
451 FRRVLTIVQA HCKLGLTATL VREDDKIVDL NFLIGPKLYE ANWMELQNNG
501 YIAKVQCAEV WCPMSPEFYR EYVAIKTKKR ILLYTMNPNK FRACQFLIKF
551 HERRNDKIIV FADNVFALKE YAIRLNKPYI YGPTSQGERM QILQNFKHNP
601 KINTIFISKV GDTSFDLPEA NVLIQISSHG GSRRQEAQRL GRVLRAKKGM
651 VAEEYNAFFY SLVSQDTQEM AYSTKRQRFL VDQGYSFKVI TKLAGMEEED
701 LAFSTKEEQQ QLLQKVLAAT DLDAEEEVVA GEFGSRSSQA SRRFGTMSSM
751 SGADDTVYME YHSSRSKAPS KHVHPLFKRF RK
```

Fig. S4. Sequence coverage of XPB by MS experiment. The matched peptides are colored in red.

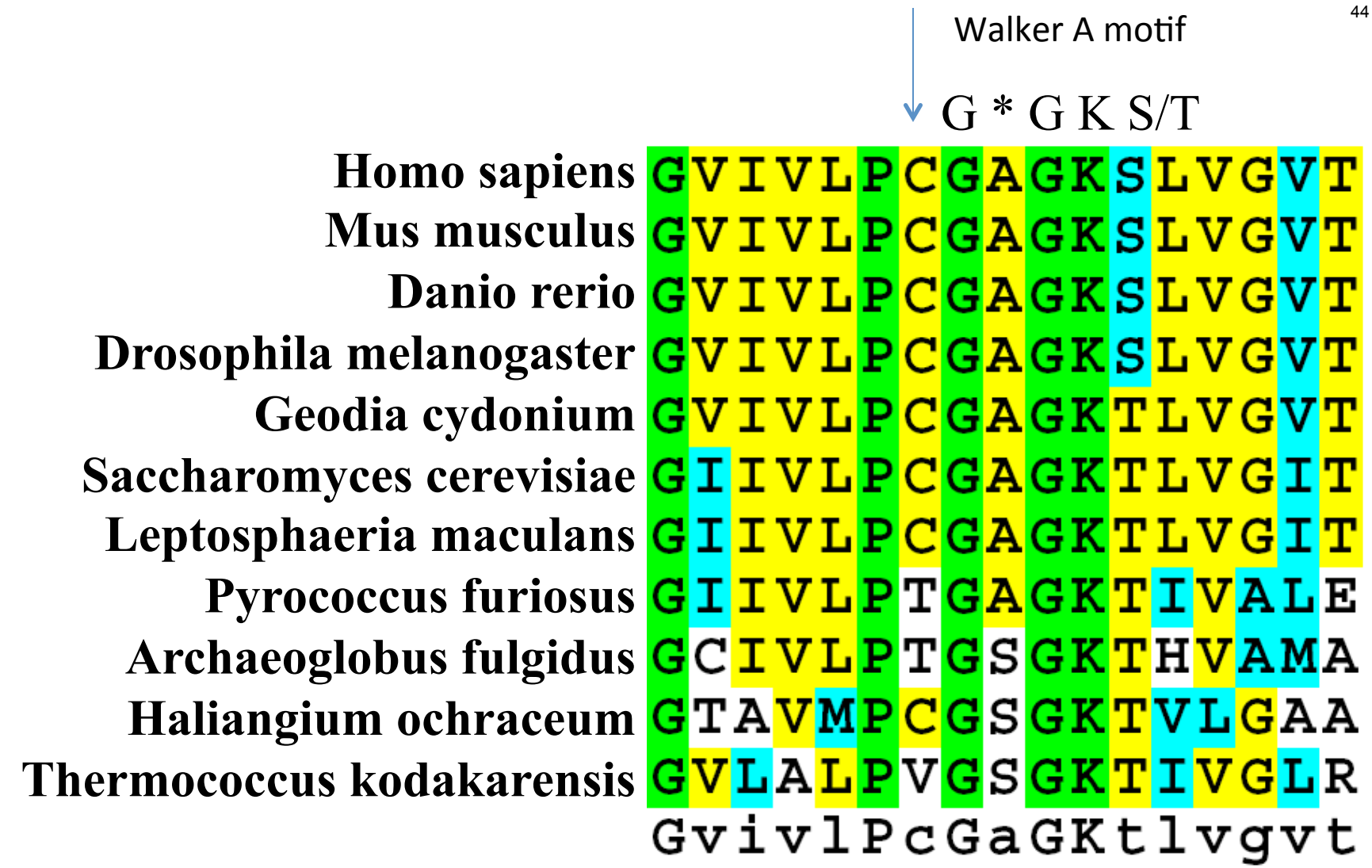

Fig. S5. Sequence alignment of XPB orthologues from different organisms. Arrow points to Cys342 in human XPB.

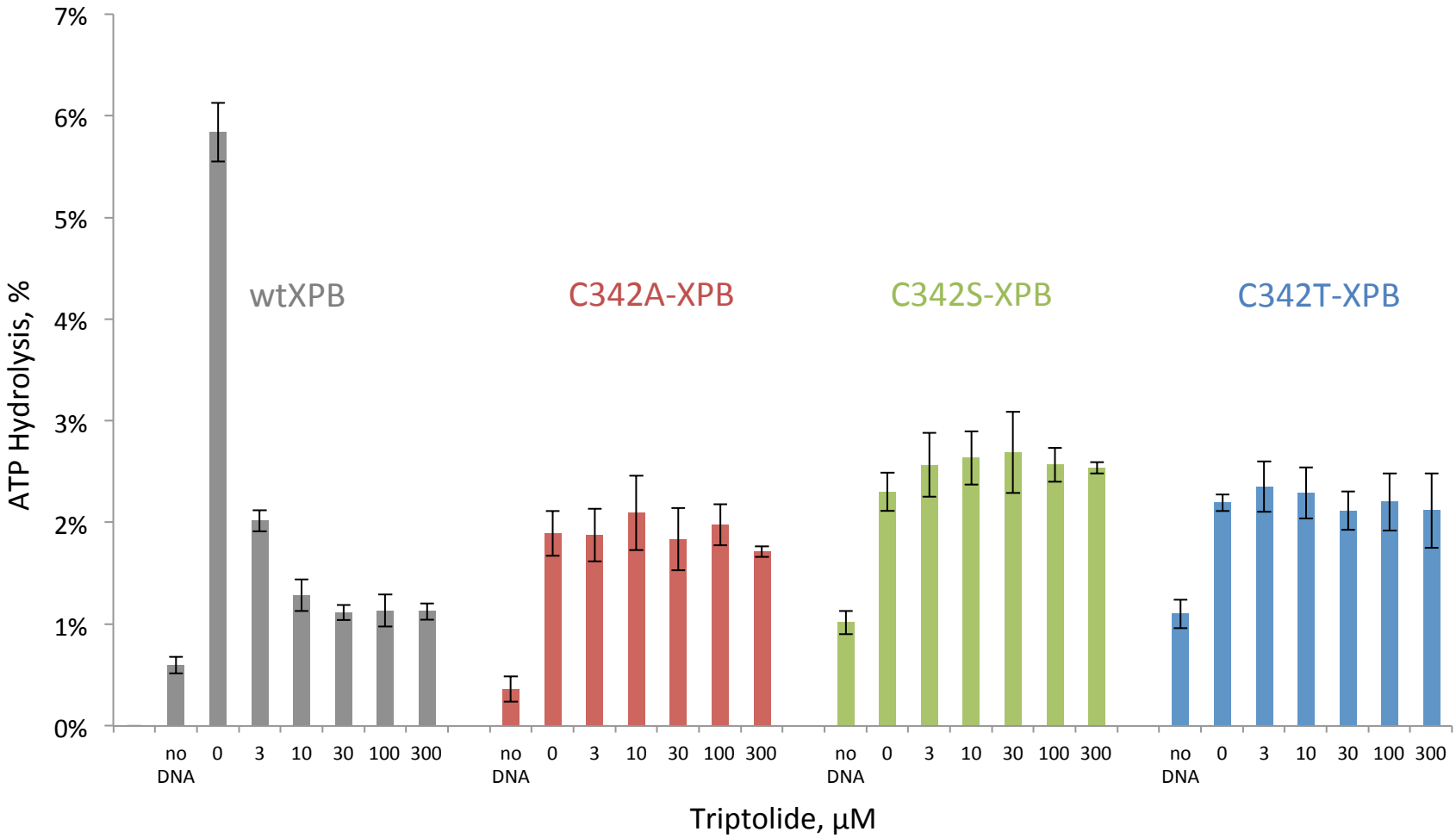

Fig. S6. TPL does not inhibit the ATPase activity of recombinant C324A, C342S,C342T-XPB mutants.

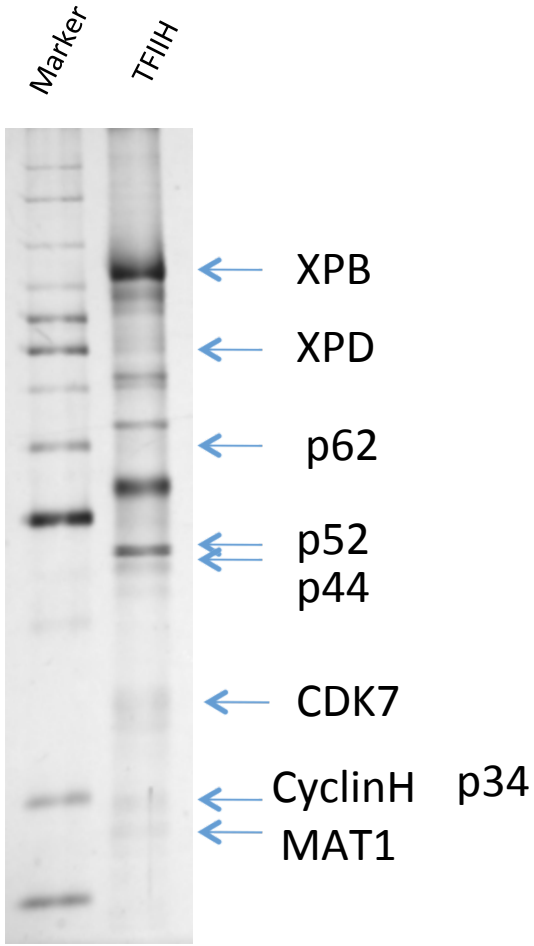

Fig. S7. Protein composition of representative TFIIH complexes with XPB mutant purified using STAP tag affinity pulldown.

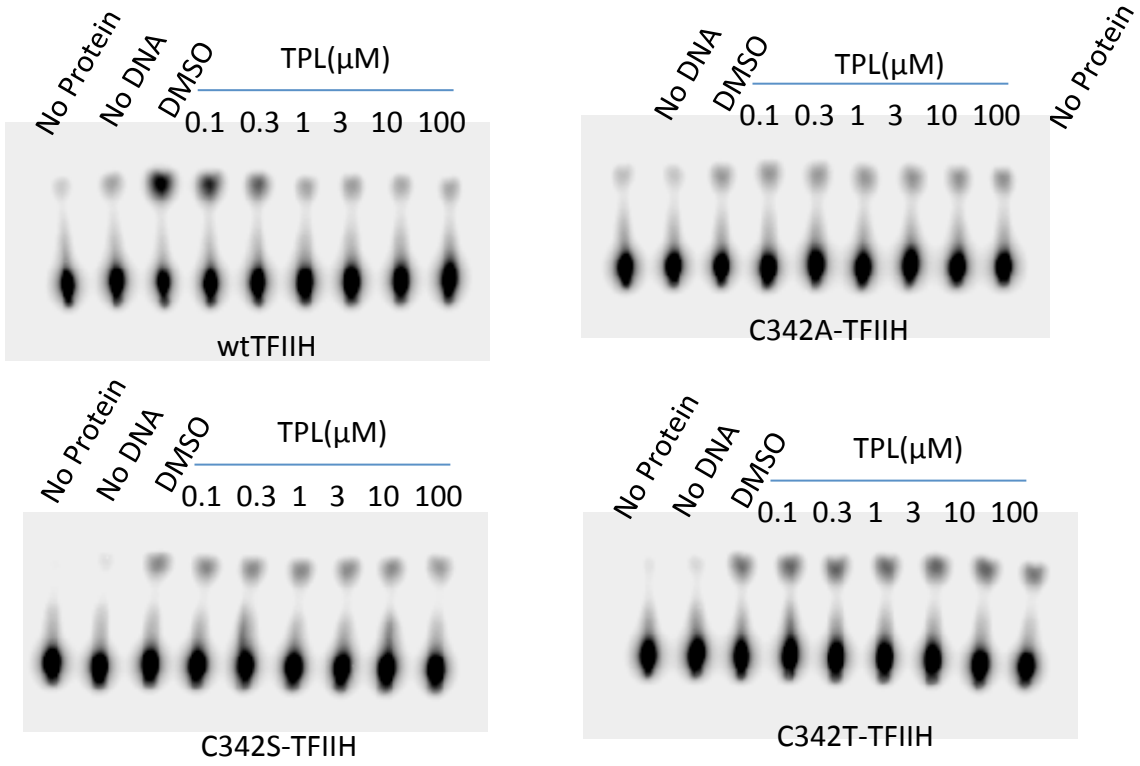

Fig. S8. Effects of triptolide on the ATPase activity of the C324A, C342S,C342T-XPB TFIIH mutants.

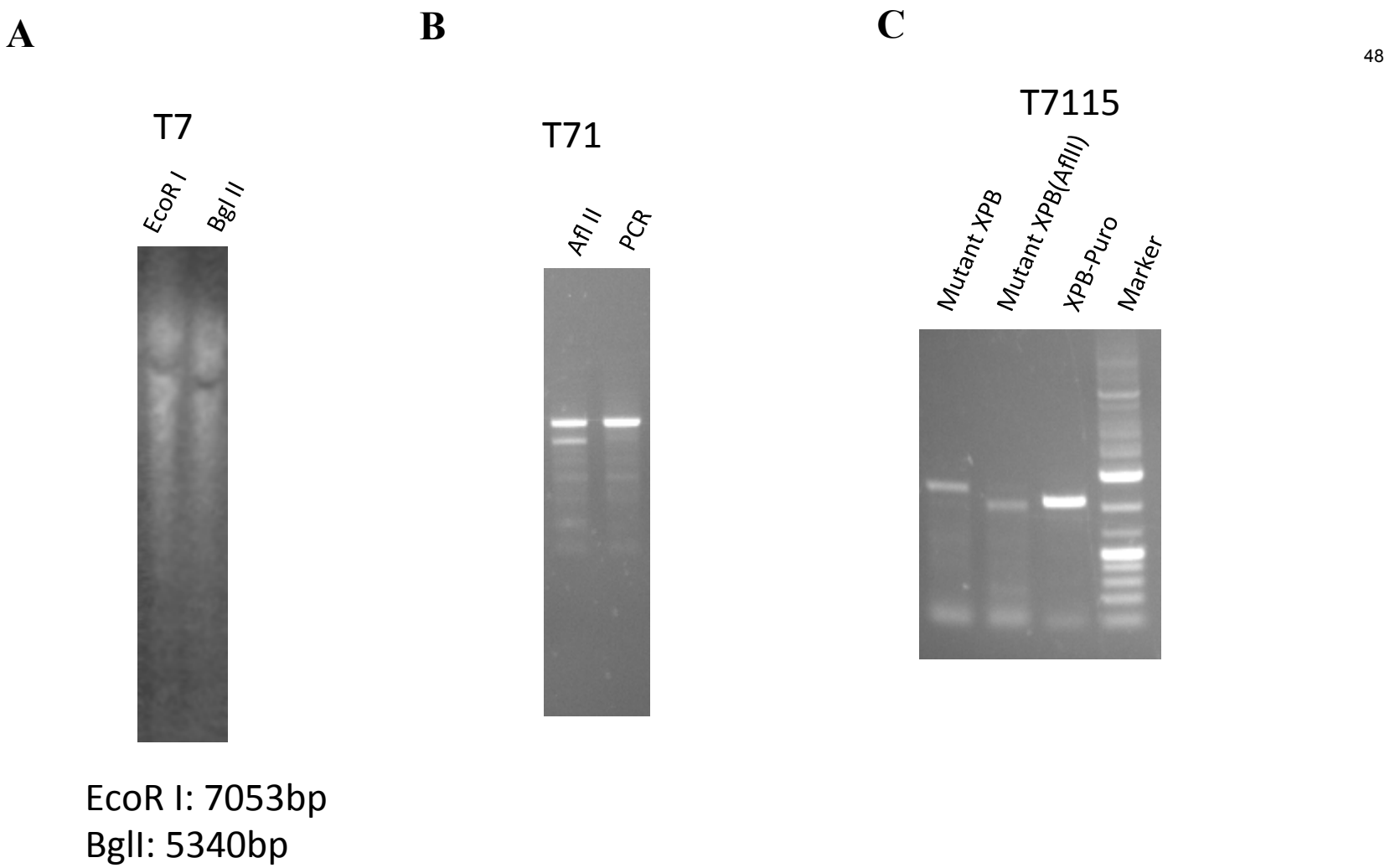

Fig. S9. Verification of the knock-in cell line T7115. (*A*) Southern blot analysis of T7 cell line that has one wild-type XPB with the other allele knocked out. (*B*) PCR-Afl II digestion analysis of T71 cell line that has one wild-type XPB and one C342T knock-in XPB. (*C*) PCR-Afl II digestion analysis of T7115 cell line with one XPB allele knocked out and the other replaced by C342T mutant. The XPB-puro lane shows that the puromycin-resistant gene was inserted the right position.

Fig. S10. Sequence confirmation of HEK293T and T7115 cell lines at the C342 coding loci. The TAAA was changed to TTAA transposon-excised site without changing the amino acid, and Cys342(TGC) was mutated to Thr342(ACC).

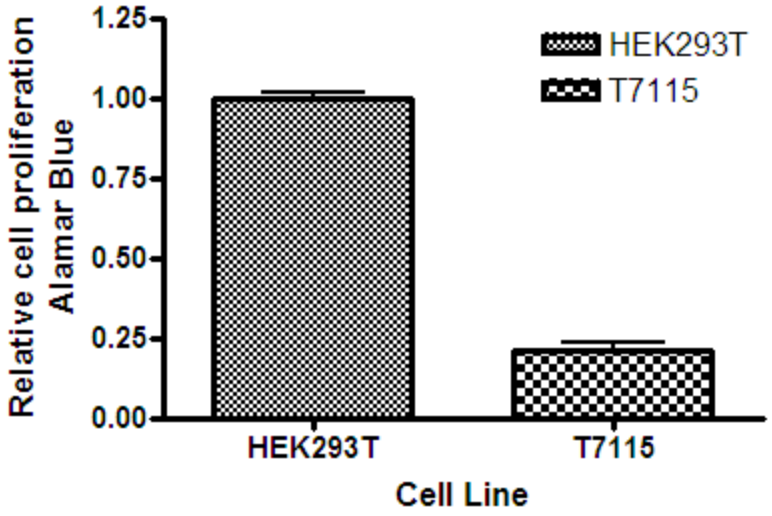

Fig. S11. The T7115 cell line has a slower proliferation than the wild type HEK293T cells. The cell proliferation was detected by Alamar Blue after three days of seeding the two different cell lines with 1000 cells per well in 96 wells plate.

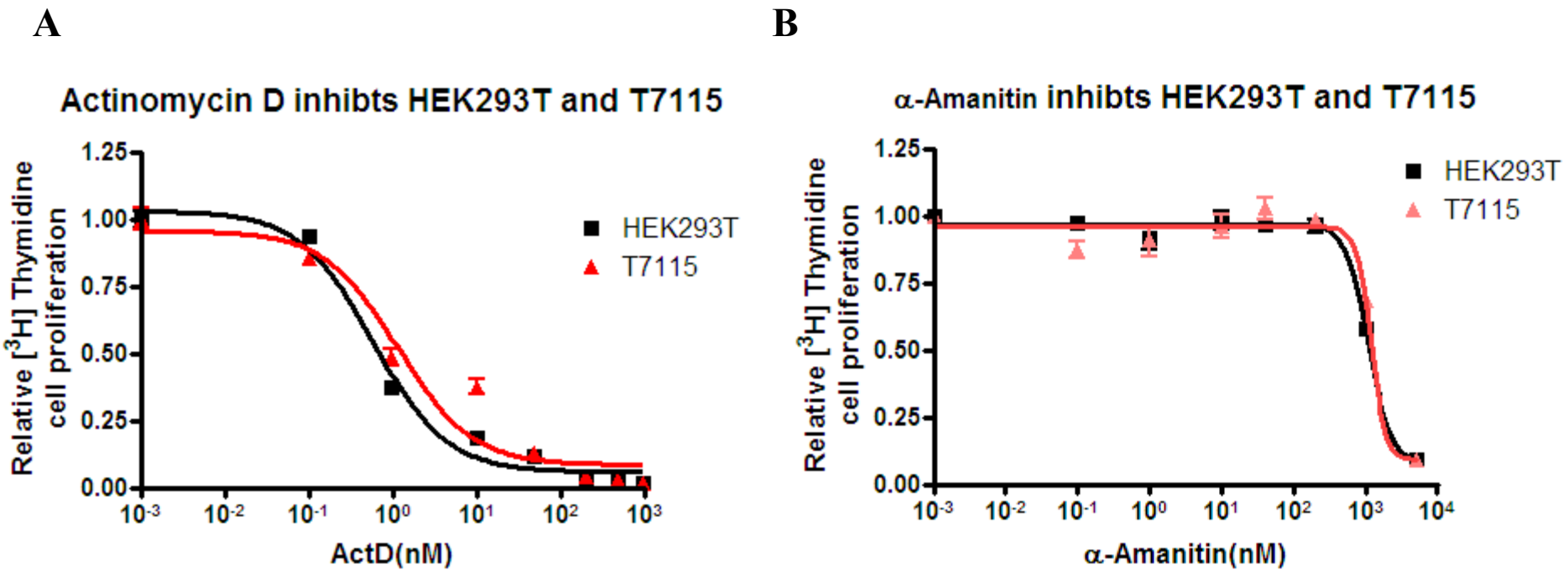

Fig. S12. The T7115 cell line is equally sensitive to Actinomycin D and α-Amanitin as wild type HEK293T cells.

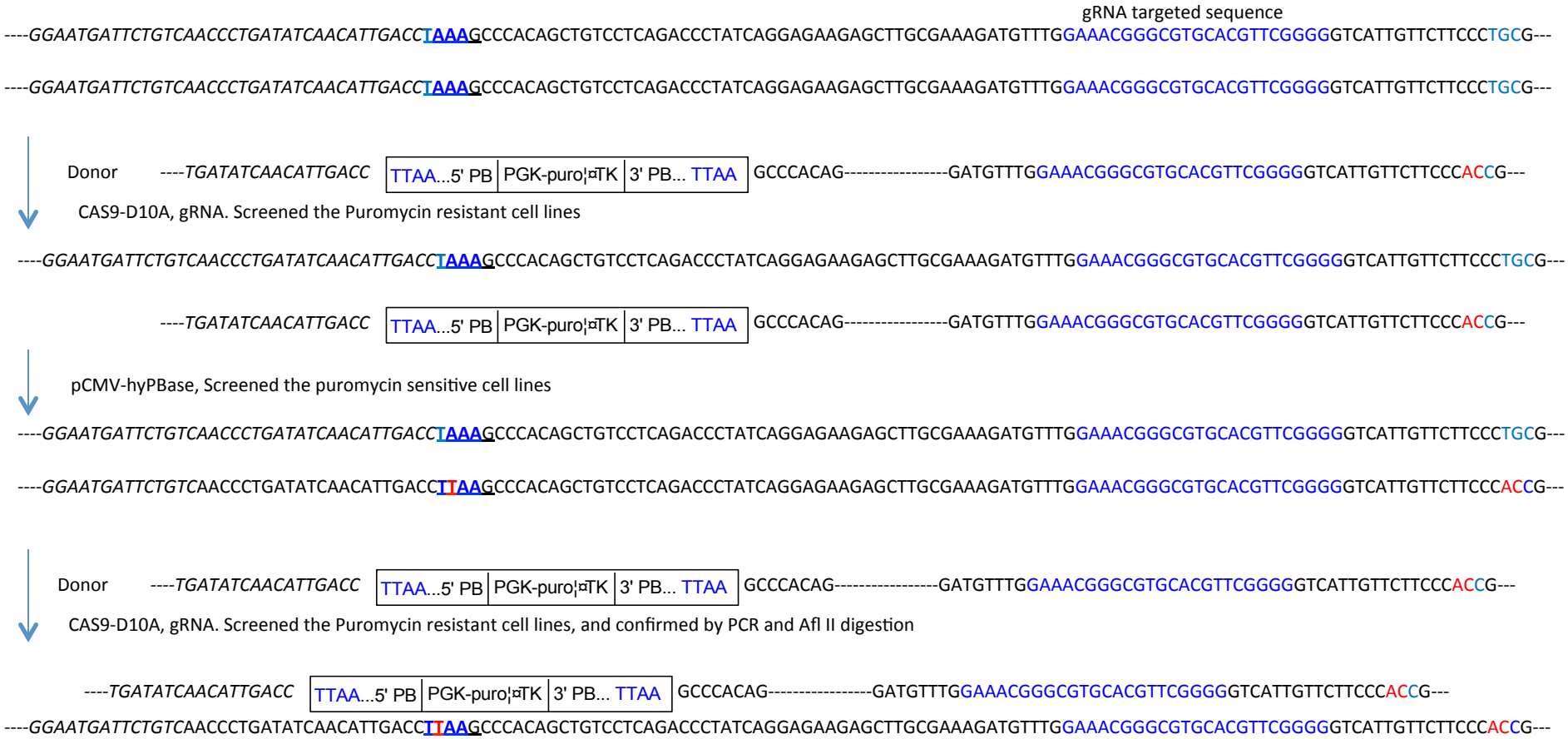

Fig. S13. The workflow to generate the C342T knock-in cell line using a combination of *CRISPR/Cas9* and *piggyBac* transposase technology