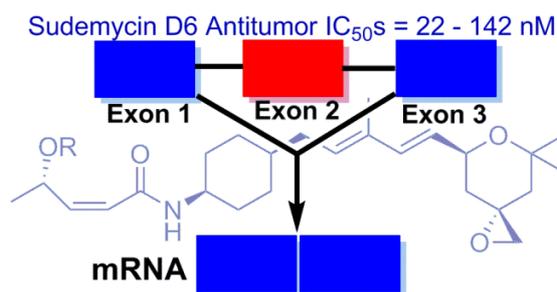


## Supporting Information

### Optimization of Antitumor Modulators of Pre-mRNA Splicing

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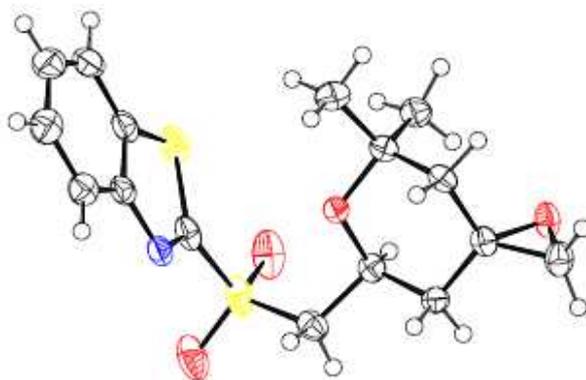
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## General Aspects:

All materials and reagents were used as is unless otherwise indicated. Air- or moisture-sensitive reactions were carried out under a nitrogen atmosphere. THF, toluene, acetonitrile, *N,N*-dimethyl formamide and  $\text{CH}_2\text{Cl}_2$  were distilled before use. All compounds were purified on Biotage pre-packed silica gel columns. TLC analysis was performed using glass TLC plates (0.25 mm, 60 F-254 silica gel). Visualization of the developed plates was accomplished by staining with ethanolic phosphomolybdic acid, ceric ammonium molybdate, or ethanolic ninhydrin followed by heating on a hot plate (120 °C). All compounds for biological assays possessed a purity of >95% as determined by ultra-high pressure liquid chromatography on a Waters Acquity UPLC/PDA/ELSD/MS system carried out with a BEH C18 2.1 x 50 mm column using gradient elution with stationary phase: BEH C18, 1.7 mm, solvents: A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile. NMR spectra were obtained on Bruker Avance II 400 MHz. The values  $d_{\text{H}}$  7.26 and  $d_{\text{C}}$  77.0 ppm were used as references for NMR spectroscopy in  $\text{CDCl}_3$ . The coupling constants deduced from  $^1\text{H}$  NMR data were obtained by first-order coupling analysis. Analytical and/or preparative SFC (Supercritical Fluid Chromatography) systems (AD-H) were used for analysis and purification of final chiral compounds. IR spectra were collected using a Nicolet IR 100 (FT IR). FT IR analyses were prepared neat or as neat films on KBr plates, and the data are reported in wave-numbers ( $\text{cm}^{-1}$ ) unless specified otherwise. Melting points (mp) were obtained on a Buchi apparatus and are uncorrected. The University of Illinois Mass Spectroscopy Laboratories and High Throughput Analytical Center at St. Jude Children's Research Hospital collected the high resolution mass spectral data.



## Supporting Figure S1: The single crystal X-ray structure of compound 5

This structure has been registered with the CCDC under the following registration number: 807948. Detailed X-ray crystallographic data is available from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

<http://www.ccdc.cam.ac.uk/pages/Home.aspx>

**(3R,5S)-butyl-7,7-dimethyl-1,6-dioxaspiro[2.5]octane-5-carboxylate(7).** Trimethylsulfoxonium iodide (788 mg, 3.51 mmol) and *t*-BuOK (0.310 mL, 2.76 mmol) were added to a 25 mL 2-neck round bottom flask. Anhydrous DMSO (5 mL) was added to the reaction flask which was under N<sub>2</sub> at room temp. The mixture was stirred for 1hr and then cooled to 15 °C. A stirred solution of keto ester **6** (500 mg, 2.190 mmol) in 3 mL anhydrous DMSO was added to the reaction flask dropwise. After 30 min the reaction mixture was quenched with ice water (5 mL). The product was then extracted with EtOAc (3 x 20 mL). The organic layers were combined, washed with water (20 mL) and brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. This was filtered, concentrated and chromatographed using a 5-30 % gradient of EtOAc/hexanes to yield 403 mg (76 %) of epoxide **7** as an oil. The analytical data for this compound were identical to that reported in our earlier publication.<sup>1</sup>

**7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)methanol (8).** In a 500 mL round bottom flask, the ester **7** (34.5 g, 141 mmol) and polymethylhydrosiloxane (PMHS) (22.3 g, 356 mmol) were combined, allowed to stir and cooled with an ice-bath. Ti(O<sup>*i*</sup>Pr)<sub>4</sub> (43 mL, 141 mmol) was added via an addition funnel to the above neat solution over 30 min and allowed to stir for 24 h at rt. The reaction mixture was diluted with THF (400 mL) and transferred to a 1 L round bottom flask, which was cooled with a cold water bath. This was then allowed to rapidly stir and slowly treated with 4M aqueous NaOH (114 mL). The mixture was allowed to stir until the evolution of gas had ceased and then for an additional 2 h at rt. The resulting cloudy white mixture was then extracted with ether (2x 550 mL, 2x300 mL 30% EtOAc in ether). The combined organic layers were then washed with water (150 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration and purification of the residue on silica gel (340g, 7-40% acetone in hexane) gave 22 g (88%) of alcohol **8** as an oil. [α]<sub>D</sub><sup>24</sup> +56.2° (c = 2.2, CHCl<sub>3</sub>). All analytical data for this compound were identical to that reported in our earlier publication.<sup>1</sup>

**tert-butyl ((1s,4s)-4-(2-hydroxyethyl)cyclohexyl)carbamate (10).** A stirred solution of 2-((1s,4s)-4-((tert-butoxycarbonyl)amino)cyclohexyl)acetic acid (15.6.0 g, 60.3 mmol) in anhyd THF (250 mL) was cooled in an ice-bath as a solution of BH<sub>3</sub>·THF (158 mL, 65.3 mmol, 1.0 M in THF) was added dropwise over 1h. The resulting homogenous solution was stirred for 3h at rt at which time the TLC indicated that the acid has been consumed. The reaction was cooled in an ice-bath and aqueous 3N NaOH (52 mL) was added to destroy the excess borane. The resulting mixture was allowed to stir for an additional 1.5h at rt. The solvent was removed under reduced pressure and the resulting residue was diluted with EtOAc (200 mL) and saturated aqueous NaHCO<sub>3</sub> (80 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2x200 mL). The combined organic layers were washed with water (200 mL) and brine (150 mL), and then dried over MgSO<sub>4</sub>. This was concentrated and the resulting viscous solid was crystalized using methylene chloride and hexane to give 12.2 g (86%) of alcohol **10** as a white solid. Melting point = 78-79 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.65 (s, 1H), 3.74 (bs, 1H), 3.71 (dd, 3H, *J* = 6.4, 11.6 Hz), 1.58 (m, 8H), 1.47 (s, 9H), 1.25 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 155.2, 79.1, 60.8, 46.4, 38.4, 29.7, 28.6, 27.8; IR (Neat) 3424, 3308, 2929, 2858, 1674, 1531, 1448, 1395, 1318, 1249, 1162, 1049 cm<sup>-1</sup>; HRMS (ESI) *m/z* Calcd for C<sub>13</sub>H<sub>27</sub>NO<sub>3</sub> (M+H)<sup>+</sup> 244.1913, found 244.1916.

**(S,Z)-4-((tert-butyl)dimethylsilyloxy)-N-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)pent-2-enamide (16: R=TBS).** The azide diene **15** (3.3 g, 9.57 mmol) was concentrated from benzene (2x20 mL), dissolved again in anhyd benzene (44 mL) and treated with Ph<sub>3</sub>P (4.5 g, 17.2 mmol) at rt. The reaction solution was degassed with nitrogen and heated at 55 °C for 1.5 h by which time LC/MS showed that all starting material had been consumed. Water (1.6 mL) was added and the reaction mixture heated again at 55 °C for another 1.5 h by which time LC/MS showed the

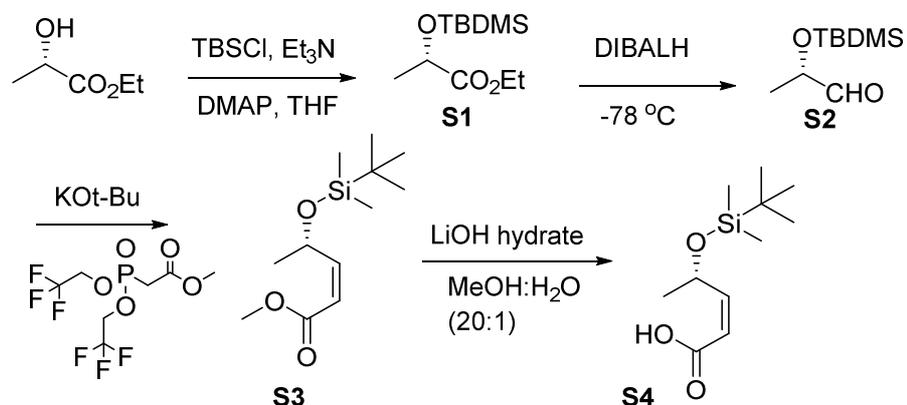
formation of free amine. The reaction mixture was cooled to rt and diluted with a mixture of CH<sub>2</sub>Cl<sub>2</sub> and ether (8:2, 150 mL). This mixture was shaken vigorously, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The resulting amine intermediate was used in the next step without further purification.

A stirred solution of crude amine in acetonitrile (45 mL) was treated with *N,N'*-diisopropylethylamine (6.6 mL, 37.3 mmol) and a solution of (S,Z)-4-((TBS)oxy)pent-2-enoic acid (3 g, 13.1 mmol) in acetonitrile (22 mL). This mixture was cooled in an ice-bath and HBTU (3.1 g, 12.3 mmol) was added portion wise to the reaction solution. The reaction suspension was stirred for 30 min at 0 °C warmed to rt, stirred another 2h at rt and then diluted with EtOAc (200 mL). After successive washing with saturated aqueous NaHCO<sub>3</sub> solution (50 mL), saturated aqueous NH<sub>4</sub>Cl solution (50 mL), saturated NaHCO<sub>3</sub> solution (50 mL) and brine (50 mL), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated and the residue was mixed with 18% EtOAc in hexane (30 mL) and the resulting solids were filtered through a sintered glass funnel. The solids were then washed successively with 18% EtOAc in hexane (50 mL) and then hexane (50 mL). The filtrate was concentrated and the residue was purified on silica gel (4-40% EtOAc in hexane) to give 4.3 g (83%) of amide **16** as a viscous liquid. All analytical data for this compound were identical to that reported in our earlier publication.<sup>1</sup>

**(S,Z)-N-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)-4-hydroxypent-2-enamide (16: R= H)**. A solution of TBS protected amide **16** (4.1 g, 7.7 mmol) in THF (37 mL) was cooled in an ice-bath as TBAF (9.2 mL, 9.2 mmol, 1.0 M in THF) was slowly added using a syringe for 5 minutes. The light yellow solution was allowed to stir for 30 min at 0 °C and after that stir for an additional 2h at rt by which time TLC and LC/MS showed the starting material has been consumed. THF was evaporated and the resulting residue was diluted with EtOAc (200 mL), water (30 mL) and saturated aqueous NaHCO<sub>3</sub> (30 mL). The organic layer was separated, washed with water (50 mL) and brine (50 mL) then dried over Na<sub>2</sub>SO<sub>4</sub>. This was concentrated and the crude residue was purified using an SFC-80 with a AD-H column (20% MeOH) to give 2.6 g (81%) of title compound as a viscous oil. All analytical data for this compound were identical to that reported in our earlier publication.<sup>1</sup>

**(S,Z)-5-(((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl (4-nitrophenyl) carbonate (17)**. A stirred solution of alcohol **16:R=H** (2 g, 4.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (70 mL) was stirred at rt as triethylamine (2.6 mL, 19.1 mmol) was added. After 5-10 min, the reaction was cooled with an ice-bath and *p*-nitrophenylchloro formate (2 g, 9.8 mmol) was added. After 10 min of stirring at 0 °C, TLC and LC/MS indicated that all of the starting material had been consumed. Cold water (100 mL) was added to the reaction mixture and diluted with methylene chloride (100 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (2x75mL). The combined organic layers were washed with water (100 mL), brine (90 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation and purification of the residue on silica gel (100 g, 10-50% EtOAc in hexane) gave 2.5 g (90%) of carbonate **17** as a fluffy solid. All analytical data for this compound were identical to that reported in our earlier publication.<sup>2</sup>

### Scheme S1



(*S,Z*)-4-((*tert*-butyldimethylsilyl)oxy)pent-2-enoic acid

**(S)-ethyl 2-((*tert*-butyldimethylsilyl)oxy)propanoate (S1).** A stirred solution of (*S*)-ethyl 2-hydroxypropanoate (120 g, 1.0 mol) in THF (1.5 L) at rt, was treated sequentially with DMAP (12.4 g, 0.1 mol) and triethylamine (1.5 L, 2.0 mol). The resulting solution was cooled in an ice-bath and was allowed to stir for 10 min. TBSCl (205 g, 1.3 mol) was added portionwise to the above stirred solution over 30 minutes. The resulting white suspension was allowed to warm to rt and then allowed to stir overnight. The reaction mixture was filtered through a sintered glass funnel to remove salts and washed with *tert*-butyl methyl ether (1.4 L). The filtrate was divided into two 700 mL portions which were each washed with 15% cold AcOH (500 mL), water (500 mL), saturated aqueous NaHCO<sub>3</sub> (350 mL), water (500 mL), brine (400 mL), and dried over MgSO<sub>4</sub>. This was filtered; the combined filtrates were concentrated and the resulting residue was purified on a silica column (2% *t*-Butyl methyl ether/hexane) to give 200 g (85%) of silyl ether **S1** as a colorless oil. All analytical data for this compound were identical with the reported data.<sup>3</sup>

**(S)-2-((*tert*-butyldimethylsilyl)oxy)propanal (S2).** A stirred solution of ester **S1** (100 g, 430 mmol) in hexane (600 mL) in a two neck round bottom flask (2L) was cooled in a -78 °C bath. This was treated with a solution of diisobutylaluminum hydride (572 mL, 1M in hexane, 572 mmol) dropwise using an additional funnel. The internal temperature was monitored and not allowed to exceed -71 °C. The resulting suspension was stirred for an additional 1h in the -78 °C bath. Water (200 mL) was added dropwise at -78 °C and this was allowed to stir for 10 minutes after which time the reaction mixture was allowed to warm to rt. *Tert*-butyl methyl ether (400 mL) was added and the resulting mixture was filtered to remove the salts and the residue was then washed with ether (2 x 200 mL). The aqueous layer was extracted with ether (2 x 200 mL). All organic layers were combined and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was concentrated under reduced pressure (bath temp. set to 30 °C and vacuum set to 200 mbar) and the resulting crude product was chromatographed on silica gel (1:1 CH<sub>2</sub>Cl<sub>2</sub>/hexane) to give 81 g (87%) of pure aldehyde **S2** as an oil. All analytical data for this compound were identical with the reported data in literature.<sup>3</sup>

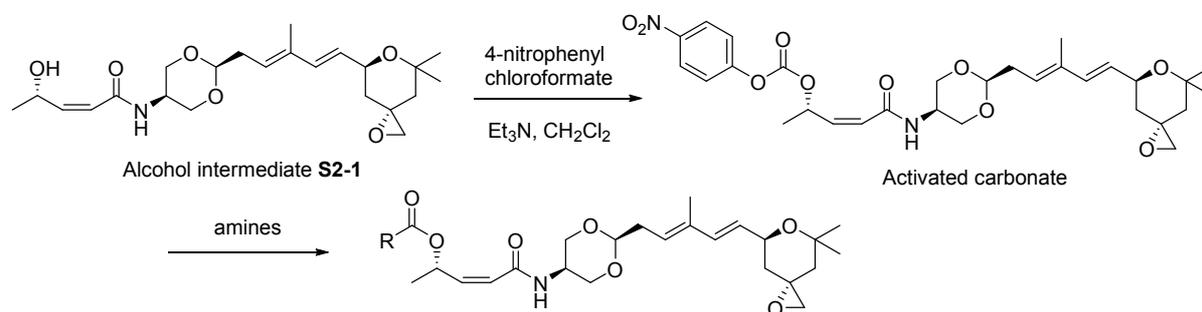
**(S,Z)-methyl 4-((*tert*-butyldimethylsilyl)oxy)pent-2-enoate (S3).** A solution of potassium *t*-butoxide (54 g, 481 mmol) in anhyd THF (800 mL) was cooled in a dry ice-bath. After 10 min, methyl methyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)acetate (161 g, 481 mmol) in THF (200 mL) was added dropwise to the reaction mixture over 1 h. The reaction mixture was allowed to stir at -78 °C (bath temp) for 1 h after which the aldehyde **S2** (686.7 g, 460 mmol) in THF (50 mL) was slowly added and then allowed to stir for an additional 2 hrs. The reaction mixture was

quenched with cold water (500 mL) and diluted with tert-butyl methyl ether (300 mL). The aqueous layer was separated and extracted again with tert-butyl methyl ether (1x500 mL, 1x400 mL). The combined organic layers were washed with water (400 mL), brine (400 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was concentrated and the residue was purified on a silica column (2% tert-butyl methyl ether in hexane) to give 68 g (61%) of ester **S3** as an oil. All analytical data for this compound were identical with the reported data in literature.<sup>4</sup>

**(S,Z)-4-((tert-butyldimethylsilyl)oxy)pent-2-enoic acid (S4)**. An ice cooled stirred solution of methyl ester **S3** (21.1 g, 87 mmol) in MeOH:H<sub>2</sub>O (420 mL, 20:1) was treated with LiOH hydrate (18.2 g, 434 mmol). The reaction suspension was allowed to warm to rt and then allowed to stir for 40 h. The reaction mixture was concentrated under reduced pressure at 25 °C until half of the solvent had evaporated. The resulting solution was cooled in an ice bath and acidified to ~pH 5 using 1 N citric acid (~170 mL) and extracted with EtOAc (400 mL, 2x100 mL). The organic fractions were combined, washed with brine and dried over MgSO<sub>4</sub>. The solvent was evaporated to give 17 g (89%) of acid derivative **S4** as a viscous oil. All analytical data for this compound were identical with the reported data in literature.<sup>5</sup>

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl pivalate (19a)**. A stirred ice cooled solution of alcohol **S2-1**<sup>2</sup> (10 mg, 0.02 mmol) in dichloromethane (1 mL) was treated with Et<sub>3</sub>N (17 μL, 0.11 mmol) and DMAP (0.58 mg, 4.74 μmol). After 5 min, pivaloyl chloride (8.7 μL, 0.07 mmol) was added. The solution was allowed to stir for 30 min at the same temperature. The reaction mixture was diluted with water (4 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2x10 mL). The organic layer was washed with saturated aqueous NaHCO<sub>3</sub> (5 mL) and brine (5 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated and the residue was purified on silica (30% EtOAc in hexane) to give 9 mg (75%) of **19a**, a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.22 (d, *J* = 8.4 Hz, 1H), 6.27 (dt, *J* = 15.7, 0.9 Hz, 1H), 6.09 (p, *J* = 6.6 Hz, 1H), 5.89 – 5.76 (m, 2H), 5.60 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.53 – 5.44 (m, 1H), 4.58 (t, *J* = 5.3 Hz, 1H), 4.50 – 4.41 (m, 1H), 4.03 – 3.88 (m, 5H), 2.57 (s, 2H), 2.48 (dd, *J* = 7.2, 5.4 Hz, 2H), 2.03 – 1.84 (m, 2H), 1.74 (d, *J* = 1.3 Hz, 3H), 1.39 (s, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.28 (s, 3H), 1.19 (s, 9H), 1.18 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 178.10, 164.83, 142.52, 135.65, 135.62, 127.99, 125.65, 123.16, 102.19, 73.05, 70.22, 70.20, 69.47, 68.59, 55.67, 51.05, 43.72, 42.46, 38.62, 38.58, 34.16, 31.53, 27.13, 23.77, 19.94, 12.64; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>44</sub>NO<sub>7</sub> (M+H)<sup>+</sup> 506.3118, found 506.3113.

#### Scheme S2



**General procedure for preparation of compounds in Table 1 (19b to 19o) from the alcohol derivative 16<sup>2</sup>**: A solution of alcohol derivative **S2-1**<sup>2</sup> (2.4 g, 5.69 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (90 mL) was stirred at room temperature as triethylamine (3.1 mL) was added. After 5-10 min, the reaction

was cooled with an ice-bath and *p*-nitrophenylchloro formate (2.3 g, 11.3 mmol) was then added portionwise. After 10 min of stirring at 0 °C, TLC and SFC showed that most of the starting material had been consumed. Cold water (100 mL) and methylene chloride (100 mL) were added. The aqueous layer was extracted with methylene chloride (2x75mL). The combined organic solvents were washed with water (100 mL), brine (90 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation and purification of the residue on silica gel (100 g, 10-50% EtOAc in hexane) gave 2.8 g (84%) of (S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl (4-nitrophenyl) carbonate as a viscous solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29 – 8.23 (m, 2H), 7.42 – 7.36 (m, 2H), 6.69 (d, *J* = 8.5 Hz, 1H), 6.49 – 6.37 (m, 1H), 6.27 (d, *J* = 15.8 Hz, 1H), 6.07 (dd, *J* = 11.5, 7.9 Hz, 1H), 5.89 (dd, *J* = 11.6, 1.2 Hz, 1H), 5.60 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.46 (t, *J* = 7.2 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 4.46 (ddd, *J* = 11.3, 6.6, 1.8 Hz, 1H), 4.00-3.90 (m, 5H), 2.58 (d, *J* = 4.8 Hz, 2H), 2.47 – 2.45 (t, *J* = 6.0 Hz, 2H), 2.03 – 1.86 (m, 2H), 1.73 (d, *J* = 0.8 Hz, 3H), 1.54 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 1.28 (d, *J* = 2.1 Hz, 3H), 1.24 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 164.22, 155.56, 151.75, 145.33, 142.22, 135.78, 135.48, 128.19, 125.30, 125.26, 123.59, 121.77, 102.29, 74.10, 73.08, 70.19, 69.46, 55.66, 51.07, 43.78, 42.45, 38.58, 34.13, 31.53, 23.77, 19.91, 12.64; HRMS (ESI) *m/z* Calcd for C<sub>30</sub>H<sub>38</sub>N<sub>2</sub>O<sub>10</sub> (M+Na)<sup>+</sup> 609.2424, found 609.2424. A stirred solution of the above activated carbonate (0.1 mmol) in dichloroethane (2 mL) was cooled in an ice-bath and the appropriate amine (3 mmol) was added dropwise. The resulting yellow solution was allowed to stir at ambient temperature until TLC indicated completion of the reaction. The solvent was then removed under reduced pressure. The yellow viscous crude was dissolved in a minimum volume of methylene chloride and treated with hexane. The resulting yellow solids were filtered and washed with 5% methylene chloride in hexane (5 mL). The filtrate was concentrated and purified on silica gel to give the corresponding carbamate derivative.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl butylcarbamate (19b).** Yield: 5.3 mg (75%) of **19b** as an oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.73 (d, *J* = 8.1 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.97 – 5.77 (m, 3H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.51 (t, *J* = 7.2 Hz, 1H), 4.75 (bt, *J* = 7.3 Hz, 1H), 4.60 (t, *J* = 5.2 Hz, 1H), 4.50 – 4.41 (m, 1H), 4.07 – 3.88 (m, 5H), 3.15 (q, *J* = 6.7 Hz, 2H), 2.57 (s, 2H), 2.49 (dd, *J* = 7.2, 5.3 Hz, 2H), 2.02 – 1.86 (m, 2H), 1.73 (d, *J* = 1.3 Hz, 3H), 1.51 – 1.41 (m, 2H), 1.40 (s, 3H), 1.37-1.31 (m, 5H), 1.28 (s, 3H), 1.27 – 1.12 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.18, 156.28, 141.59, 135.66, 135.50, 127.82, 125.82, 123.61, 102.06, 73.07, 70.12, 69.46, 68.85, 55.69, 51.07, 43.85, 43.46, 42.46, 40.71, 38.60, 34.16, 32.00, 31.54, 23.77, 20.39, 19.93, 13.75, 12.60; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 521.3227, found 521.3220.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl diethylcarbamate (19c).** Yield: 4.2 mg (62%) of **19c** as an oil. <sup>1</sup>H NMR (400 MHz, Chloroform-d) δ 7.92 (d, *J* = 8.3 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.96 – 5.87 (m, 1H), 5.86 – 5.77 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.3 Hz, 1H), 4.58 (t, *J* = 5.3 Hz, 1H), 4.49 – 4.41 (m, 1H), 4.07 – 3.86 (m, 5H), 3.38 – 3.18 (m, 4H), 2.57 (s, 2H), 2.49 (dd, *J* = 7.3, 5.4 Hz, 2H), 2.02 – 1.85 (m, 2H), 1.73 (d, *J* = 1.3 Hz, 3H), 1.39 (s, 3H), 1.37 (d, *J* = 6.3 Hz, 3H), 1.28 (s, 3H), 1.26 – 1.13 (m, 4H), 1.11 (t, *J* = 7.1 Hz, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.28, 155.59, 141.76, 135.70, 135.50, 127.87, 125.92, 123.47, 102.05, 73.04, 70.12, 69.50, 69.08, 55.67, 51.05, 43.82, 42.46, 38.58, 34.20, 31.53, 23.76, 21.07, 20.45, 14.22, 12.60; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 521.3227, found 521.3223.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl tert-butylcarbamate (19d).** Yield: 3.8 mg (54%) of **19d** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 7.71 (s, 1H), 6.28 (dt, *J* = 15.7, 0.9 Hz, 1H), 5.92 – 5.76 (m, 3H), 5.59 (dd, *J* = 15.7, 6.5 Hz, 1H), 5.54 (t, *J* = 7.3 Hz, 1H), 4.68 (s, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 4.51 – 4.41 (m, 1H), 4.05 – 3.95 (m, 2H), 3.97 – 3.87 (m, 3H), 2.57 (s, 2H), 2.48 (dd, *J* = 7.2, 5.3 Hz, 2H), 2.02 – 1.87 (m, 2H), 1.73 (s, 3H), 1.40 (s, 3H), 1.34 (d, *J* = 5.9 Hz, 3H), 1.30 (s, 9H), 1.28 (s, 3H), 1.24 – 1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.23, 154.44, 142.09, 135.63, 135.42, 127.78, 125.82, 123.29, 102.03, 73.04, 70.02, 69.42, 68.36, 55.66, 51.03, 50.43, 43.87, 42.43, 38.59, 34.17, 31.52, 28.95, 23.75, 20.35, 12.58; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 521.3227, found 521.3232.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl methyl(propyl)carbamate (19e).** Yield: 4.2 mg (52%) of **19e** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.91 (bd, 1H), 6.27 (d, *J* = 15.8 Hz, 1H), 5.95 – 5.77 (m, 3H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.1 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 4.51 – 4.42 (m, 1H), 4.06 – 3.87 (m, 5H), 3.18 (dd, *J* = 14.2, 7.0, 2H), 2.88 (s, 3H), 2.57 (s, 2H), 2.50 (s, 2H), 2.03 – 1.86 (m, 2H), 1.73 (d, *J* = 0.8 Hz, 3H), 1.54 (dd, *J* = 14.8, 7.4 Hz, 2H), 1.40 (s, 3H), 1.37 (d, *J* = 6.2 Hz, 3H), 1.28 (s, 3H), 1.23 – 1.13 (m, 2H), 0.94 – 0.80 (m, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.23, 156.15, 141.54, 135.67, 135.45, 127.83, 125.89, 123.60, 102.01, 73.01, 70.09, 70.06, 69.47, 69.25, 55.63, 51.02, 50.54, 43.79, 42.42, 38.55, 34.17, 33.91, 31.51, 23.74, 21.10, 20.39, 12.57, 11.12; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 521.3227, found 521.3231.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl (2-methoxyethyl)carbamate (19f).** Yield: 3.6 mg (50%) of **19f** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 7.64 (d, *J* = 8.1 Hz, 1H), 6.32 – 6.23 (m, 1H), 6.00 – 5.90 (m, 1H), 5.89 – 5.78 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.3 Hz, 1H), 5.09 (t, *J* = 5.8 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 4.51 – 4.42 (m, 1H), 4.06 – 3.87 (m, 5H), 3.43 (dd, *J* = 5.5, 4.5 Hz, 2H), 3.34 (d, *J* = 0.8 Hz, 5H), 2.57 (d, *J* = 0.8 Hz, 2H), 2.49 (dd, *J* = 7.3, 5.3 Hz, 2H), 2.03 – 1.85 (m, 2H), 1.77 – 1.71 (m, 3H), 1.40 (s, 3H), 1.35 (d, *J* = 6.4 Hz, 3H), 1.28 (s, 3H), 1.24 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.12, 156.17, 141.80, 135.64, 135.53, 127.89, 125.78, 123.48, 102.07, 73.07, 71.38, 70.14, 69.46, 69.12, 58.77, 55.69, 51.07, 43.83, 42.46, 40.71, 38.59, 34.17, 31.54, 23.77, 20.36, 12.60; HRMS (ESI) *m/z* Calcd for C<sub>27</sub>H<sub>43</sub>N<sub>2</sub>O<sub>8</sub> (M+H)<sup>+</sup> 523.3019, found 523.3013.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl butyl(methyl)carbamate (19g).** Yield: 4.2 mg (46%) of **19g** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.85 (bd, 1H), 6.20 (dt, *J* = 16.1, 1.0 Hz, 1H), 5.89 – 5.67 (m, 3H), 5.52 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.43 (t, *J* = 7.3 Hz, 1H), 4.52 (t, *J* = 5.1 Hz, 1H), 4.45 – 4.30 (m, 1H), 4.01 – 3.77 (m, 5H), 3.25-3.10 (m, 2H), 2.81 (s, 3H), 2.50 (s, 2H), 2.44 (d, *J* = 6.4 Hz, 2H), 1.97 – 1.77 (m, 2H), 1.67 (d, *J* = 1.4 Hz, 3H), 1.42 (p, *J* = 7.4, 6.2 Hz, 2H), 1.33 (s, 3H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.25-1.17 (m, 5H), 1.16-1.06 (m, 2H), 0.90-0.80 (m, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.27, 156.12, 141.47, 135.68, 135.45, 127.84, 125.88, 123.58, 102.02, 73.02, 70.05, 69.47, 69.26, 55.64, 51.02, 48.30, 43.82, 42.42, 38.55, 34.36, 34.17, 31.50, 29.95, 23.74, 20.39, 19.75, 13.83, 12.56; HRMS (ESI) *m/z* Calcd for C<sub>29</sub>H<sub>47</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 535.3383, found 535.3379.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl isopropyl(methyl)carbamate (19h).** Yield: 4.5 mg (50%) of **19h** as an oil. <sup>1</sup>H NMR (400 MHz,

CDCl<sub>3</sub>) δ 7.99 (s, 1H), 6.26 (dd, *J* = 15.7, 1.1 Hz, 1H), 5.96 – 5.75 (m, 3H), 5.58 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.49 (t, *J* = 7.3 Hz, 1H), 4.58 (t, *J* = 5.3 Hz, 1H), 4.52 – 4.19 (m, 2H), 4.03 – 3.87 (m, 5H), 2.73 (s, 3H), 2.55 (s, 2H), 2.48 (t, *J* = 6.4 Hz, 2H), 2.06 – 1.82 (m, 2H), 1.72 (d, *J* = 1.3 Hz, 3H), 1.38 (s, 3H), 1.36 (d, *J* = 6.2 Hz, 3H), 1.26 (s, 3H), 1.22 – 1.12 (m, 2H), 1.08 (d, *J* = 6.6 Hz, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.26, 155.77, 141.66, 135.66, 135.45, 127.84, 125.90, 123.52, 102.00, 73.01, 70.09, 69.46, 69.20, 55.63, 51.01, 46.36, 43.78, 42.43, 38.56, 34.17, 31.51, 26.91, 23.74, 20.43, 19.83, 12.56; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 521.3227, found 521.322.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl tert-butyl(methyl)carbamate (19i).** Yield: 5.4 mg (59%) of **19i** as an oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.04 (d, *J* = 8.1 Hz, 1H), 6.27 (dt, *J* = 15.7, 1.0 Hz, 1H), 5.91 – 5.73 (m, 3H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.3 Hz, 1H), 4.58 (t, *J* = 5.3 Hz, 1H), 4.52 – 4.39 (m, 1H), 4.07 – 3.97 (m, 2H), 3.98 – 3.82 (m, 3H), 2.91 (s, 3H), 2.57 (s, 2H), 2.53 – 2.40 (m, 2H), 2.02 – 1.87 (m, 2H), 1.73 (d, *J* = 1.3 Hz, 3H), 1.41-1.36 (m, 15H), 1.28 (s, 3H), 1.23 – 1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.36, 156.19, 141.63, 135.71, 135.42, 127.82, 125.96, 123.47, 102.04, 73.02, 70.07, 70.02, 69.49, 69.08, 55.65, 55.57, 51.04, 43.86, 42.44, 38.57, 34.21, 31.52, 31.32, 28.79, 23.75, 20.42, 12.58; HRMS (ESI) *m/z* Calcd for C<sub>29</sub>H<sub>47</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 535.3383, found 535.3392.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl morpholine-4-carboxylate (19j).** Yield: 6 mg (66%) of **19j** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.68 (d, *J* = 8.4 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.99 (p, *J* = 6.5 Hz, 1H), 5.88 – 5.78 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.3 Hz, 1H), 4.59 (t, *J* = 5.3 Hz, 1H), 4.49-4.43 (m, 1H), 4.07 – 3.88 (m, 5H), 3.65 (t, *J* = 4.9 Hz, 4H), 3.46 (t, *J* = 5.0 Hz, 4H), 2.57 (s, 2H), 2.49 (dd, *J* = 7.1, 5.4 Hz, 2H), 2.03 – 1.86 (m, 2H), 1.73 (d, *J* = 1.4 Hz, 3H), 1.41 – 1.36 (m, 6H), 1.28 (s, 3H), 1.24 – 1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.00, 155.03, 141.69, 135.62, 135.54, 127.95, 125.74, 123.56, 102.06, 77.22, 73.04, 70.15, 69.77, 69.49, 55.65, 51.05, 43.77, 42.43, 38.58, 34.18, 31.52, 23.76, 20.33, 12.61; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>43</sub>N<sub>2</sub>O<sub>8</sub> (M+H)<sup>+</sup> 535.3019, found 535.3001.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl piperidine-1-carboxylate (19k).** Yield: 5 mg (55%) of **19k** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.01 (d, *J* = 8.4 Hz, 1H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.90-5.82(m, 1H), 5.83 – 5.79 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.3 Hz, 1H), 4.59 (t, *J* = 5.3 Hz, 1H), 4.50 – 4.41 (m, 1H), 4.06 – 3.88 (m, 5H), 3.40 (t, *J* = 5.6 Hz, 4H), 2.57 (s, 2H), 2.50 (t, *J* = 7.3 Hz, 2H), 2.02 – 1.87 (m, 2H), 1.73 (d, *J* = 1.4 Hz, 3H), 1.59-1.54 (m, 2H), 1.55 – 1.47 (m, 4H), 1.39 (s, 3H), 1.36 (d, *J* = 6.1 Hz, 3H), 1.27 (s, 3H), 1.24 – 1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.27, 155.12, 141.41, 135.70, 135.48, 127.84, 125.92, 123.62, 102.02, 73.02, 70.12, 69.49, 69.23, 55.65, 51.04, 44.72, 43.78, 42.43, 38.57, 34.19, 31.52, 25.73, 24.34, 23.75, 20.41, 12.59; HRMS (ESI) *m/z* Calcd for C<sub>29</sub>H<sub>45</sub>N<sub>2</sub>O<sub>7</sub> (M+H)<sup>+</sup> 533.3227, found 533.3222.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl pyrrolidine-1-carboxylate (19l).** Yield: 6.7 mg (76%) of **19l** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.06 (d, *J* = 8.3 Hz, 1H), 6.28 (d, *J* = 15.7 Hz, 1H), 5.92 – 5.84 (m, 1H), 5.84 – 5.78 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.51 (t, *J* = 7.2 Hz, 1H), 4.59 (t, *J* = 5.3 Hz, 1H), 4.50 – 4.40 (m, 1H), 4.07 – 3.86 (m, 5H), 3.35 (dt, *J* = 13.4, 6.7 Hz, 4H), 2.57 (s, 2H), 2.54 – 2.47 (m, 2H), 2.01 – 1.89 (m, 2H), 1.88-1.78 (m 4H), 1.73 (d, *J* = 1.3 Hz, 3H), 1.39 (s, 3H), 1.36 (d, *J* = 6.0 Hz, 3H),

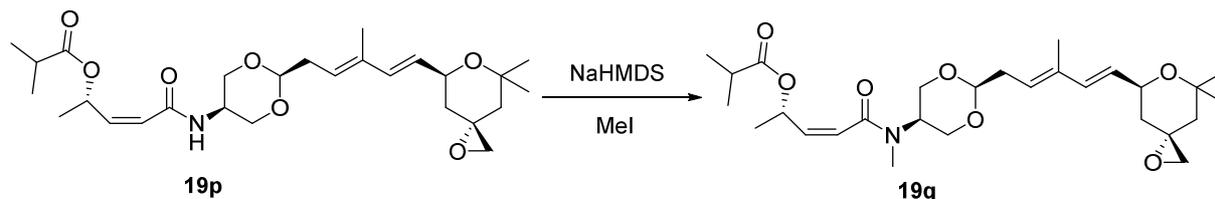
1.27 (s, 3H), 1.23 – 1.12 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.34, 154.83, 141.33, 135.73, 135.47, 127.85, 125.99, 123.73, 102.04, 73.05, 70.15, 70.12, 69.52, 68.96, 55.67, 51.06, 46.11, 45.81, 43.82, 42.45, 38.59, 34.19, 31.54, 25.75, 24.96, 23.77, 20.54, 12.60; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{28}\text{H}_{43}\text{N}_2\text{O}_7$  ( $\text{M}+\text{H}$ ) $^+$  519.307, found 519.3074.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl azetidine-1-carboxylate (19m).** Yield: 4 mg (78%) of **19m** as a viscous oil.  $^1\text{H}$  NMR (400 MHz, Chloroform- $d$ )  $\delta$  7.85 (d,  $J$  = 8.3 Hz, 1H), 6.27 (d,  $J$  = 15.7 Hz, 1H), 5.93 – 5.83 (m, 1H), 5.80 (dd,  $J$  = 4.2, 1.4 Hz, 2H), 5.59 (dd,  $J$  = 15.7, 6.6 Hz, 1H), 5.51 (t,  $J$  = 7.2 Hz, 1H), 4.59 (t,  $J$  = 5.2 Hz, 1H), 4.51 – 4.41 (m, 1H), 4.05 – 3.88 (m, 9H), 2.57 (s, 2H), 2.50 (dd,  $J$  = 7.3, 5.3 Hz, 2H), 2.26–2.18 (m, 2H), 2.05 – 1.86 (m, 2H), 1.74 (d,  $J$  = 1.3 Hz, 3H), 1.39 (s, 3H), 1.34 (d,  $J$  = 6.2 Hz, 3H), 1.27 (s, 3H), 1.24 – 1.13 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.17, 156.17, 141.25, 135.69, 135.47, 127.83, 125.90, 123.72, 102.02, 73.02, 70.10, 69.49, 68.89, 55.64, 51.03, 49.45, 43.79, 42.43, 38.57, 34.15, 31.51, 23.74, 20.41, 15.70, 12.58; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{27}\text{H}_{41}\text{N}_2\text{O}_7$  ( $\text{M}+\text{H}$ ) $^+$  505.2914, found 505.2916.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl methylcarbamate (19n).** Yield: 3 mg (52%) of **19n** as an oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J$  = 7.8 Hz, 1H), 6.27 (d,  $J$  = 15.7 Hz, 1H), 5.96 – 5.88 (m, 1H), 5.86 – 5.79 (m, 2H), 5.59 (dd,  $J$  = 15.7, 6.6 Hz, 1H), 5.52 (t,  $J$  = 7.1 Hz, 1H), 4.74 (s, 1H), 4.61 (t,  $J$  = 5.1 Hz, 1H), 4.50 – 4.42 (m, 1H), 4.06 – 3.89 (m, 5H), 2.77 (d,  $J$  = 4.9 Hz, 3H), 2.57 (s, 2H), 2.50 (t,  $J$  = 6.2 Hz, 2H), 2.07 – 1.86 (m, 2H), 1.73 (s, 3H), 1.40 (s, 3H), 1.34 (d,  $J$  = 6.3 Hz, 3H), 1.29 (s, 3H), 1.26 – 1.11 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.22, 156.78, 141.26, 135.66, 135.50, 127.77, 125.82, 123.80, 102.04, 73.09, 70.12, 69.47, 68.96, 55.70, 51.08, 43.87, 42.47, 38.59, 34.15, 31.54, 29.30, 27.47, 23.78, 20.41, 12.59; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{25}\text{H}_{39}\text{N}_2\text{O}_7$  ( $\text{M}+\text{H}$ ) $^+$  479.2757, found 479.2763.

**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl carbamate (19o).** Yield 6 mg (95%) of **19o** as a oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.76 (d,  $J$  = 8.0 Hz, 1H), 6.28 (d,  $J$  = 15.7 Hz, 1H), 5.97 – 5.87 (m, 1H), 5.86 – 5.80 (m, 2H), 5.64 – 5.50 (m, 2H), 4.74 (s, 2H), 4.61 (t,  $J$  = 4.9 Hz, 1H), 4.52 – 4.41 (m, 1H), 4.09 – 3.87 (m, 5H), 2.57 (s, 2H), 2.49 (t,  $J$  = 7.2 Hz, 2H), 2.02 – 1.86 (m, 2H), 1.74 (s, 3H), 1.39 (s, 3H), 1.37 (d,  $J$  = 6.3 Hz, 3H), 1.28 (s, 3H), 1.23 – 1.09 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.18, 156.46, 140.64, 135.73, 135.47, 127.72, 125.81, 124.03, 102.03, 73.11, 70.14, 70.08, 69.48, 69.35, 55.72, 51.09, 43.89, 42.45, 38.60, 34.10, 31.54, 23.79, 20.35, 12.61; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_7$  ( $\text{M}+\text{H}$ ) $^+$  465.2601, found 465.2610.

### Scheme S3: Preparation of N-methyl derivative (19q)



**(S,Z)-5-(((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)(methyl)amino)-5-oxopent-3-en-2-yl isobutyrate (19q).** A cooled stirred solution (-78 °C; bath temp) of amide **19p** (10 mg, 0.02 mmol) in anhyd THF (0.5 mL) was treated with a solution of NaHMDS (22  $\mu$ L, 1M in THF). After 30 min Mel (14 mg, 0.1 mmol) was added. The resulting solution was stirred for another 3 h at -78 °C (bath temp) and then allowed to stir at room temp for 4 h. Saturated aqueous NH<sub>4</sub>Cl (2 mL) was added and the product was extracted with EtOAc (2x10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude residue was purified by prep-SFC (ODH column, 10% MeOH) to give 3.6 mg (35%) of N-methylated derivative **19q** as an oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.26 (d, *J* = 15.7, 1H), 6.15 (d, *J* = 10.6, 1H), 5.83 (dt, *J* = 13.9, 8.2 Hz, 2H), 5.60 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.50 (t, *J* = 7.2 Hz, 1H), 4.62 (t, *J* = 5.1 Hz, 1H), 4.46 (dd, *J* = 9.5, 7.0 Hz, 1H), 4.33 (s, 1H), 3.35 (s, 3H), 2.57 (s, 2H), 2.52 – 2.44 (m, 2H), 2.04 – 1.85 (m, 2H), 1.74 (s, 3H), 1.40 (s, 3H), 1.38 (d, *J* = 6.2, 3H), 1.28 (s, 3H), 1.30-1.12 (m, 2H), 1.14 (d, *J* = 5.6, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  176.21, 167.09, 140.55, 135.66, 135.65, 127.93, 125.67, 123.00, 101.59, 73.05, 70.06, 69.51, 68.51, 55.68, 51.06, 45.85, 42.46, 38.59, 34.47, 34.34, 34.05, 31.54, 23.77, 19.97, 18.97, 18.94, 12.63; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>44</sub>NO<sub>7</sub> (M+H)<sup>+</sup> 506.3118, found 506.3111.

**(S,Z)-5-(((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl (2-(dimethylamino)ethyl)(methyl)carbamate (18c).** The title compound was prepared from compound **17** (25 mg, 0.04 mmol) and N<sup>1</sup>,N<sup>1</sup>,N<sup>2</sup>-trimethylethane-1,2-diamine (13 mg, 0.12 mmol) using the procedure for compound **2**. Yield: 8 mg (34%) of **18c** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.22 – 8.05 (m, 1H), 6.20 (d, *J* = 15.7 Hz, 1H), 5.82 – 5.70 (m, 1H), 5.58 – 5.39 (m, 4H), 4.45 – 4.33 (m, 1H), 4.06 (s, 1H), 3.51-3.21 (m, 2H), 2.86 (s, 3H), 2.50 (s, 2H), 2.41 – 2.32 (m, 2H), 2.22 – 2.17 (m, 6H), 2.05-1.95 (m, 2H), 1.96 – 1.80 (m, 2H), 1.73 – 1.58 (m, 5H), 1.55-1.45 (m, 4H), 1.37-1.31 (m, 4H), 1.30-1.27 (m, 2H), 1.28 (d, *J* = 6.1 Hz, 3H), 1.22 (s, 3H), 1.18 – 1.05 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.12, 156.13, 136.47, 136.26, 133.60, 132.32, 126.87, 126.15, 73.02, 69.60, 69.55, 57.17, 56.78, 55.68, 51.03, 46.86, 45.71, 45.35, 42.45, 38.64, 36.76, 34.78, 34.53, 31.53, 29.59, 29.46, 27.88, 27.59, 23.76, 20.65, 12.48; HRMS (ESI) *m/z* Calcd for C<sub>31</sub>H<sub>51</sub>N<sub>3</sub>O<sub>5</sub> (M+H)<sup>+</sup> 546.3907, found 546.3932.

**(S,Z)-5-(((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl carbamate (18d).** The title compound was prepared from compound **17** (25 mg, 0.04 mmol) and ammonia (0.85 mL, 0.42 mmol, 0.5 M in THF) by following the procedure for compound **2**. Yield: 16 mg (81%) of **18d** as a viscous oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.62 (d, *J* = 7.7 Hz, 1H), 6.28 (d, *J* = 15.7 Hz, 1H), 5.87 – 5.75 (m, 1H), 5.70 – 5.46 (m, 4H), 4.74 (s, 2H), 4.50-4.42 (m, 1H), 4.12 – 4.06 (m, 1H), 2.57 (s, 2H), 2.15 – 2.06 (m, 2H), 2.01 – 1.87 (m, 2H), 1.78-1.67 (m, 5H), 1.63-1.52 (m, 4H), 1.50-1.42 (m, 1H), 1.39 (s, 3H), 1.37 – 1.29 (m, 5H), 1.27 (s, 3H), 1.22 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.03, 156.62, 136.70, 136.35, 133.78, 131.98, 126.77, 126.03, 73.08, 69.57, 69.51, 55.68, 51.04, 45.41, 42.45, 38.58, 36.62, 34.22, 31.52, 29.52, 29.44, 27.66, 27.35, 23.77, 20.50, 12.48; HRMS (ESI) *m/z* Calcd for C<sub>26</sub>H<sub>41</sub>N<sub>2</sub>O<sub>5</sub> (M+H)<sup>+</sup> 461.3015, found 461.3020.

**General procedure for preparation of compounds 20a to 20i from alcohol derivative<sup>2</sup>:** A stirred suspension of NaH (2.5 mg, 0.063 mmol, 2.2 eq, 60% in mineral oil) in anhyd DMF at rt was treated with the alcohol derivative **S2-1**<sup>2</sup> (12 mg, 0.02 mmol, 1 eq) in DMF (0.3 mL). After 15 min, the appropriate halo pyrimidine (0.08 mmol) in DMF (0.3 mL) was added. The resulting suspension was stirred for 4h at rt. Water (3 mL) and EtOAc (5 mL) were added. The organic layer was separated and the aqueous layer was extracted with EtOAc (2x10 mL). The combined

organic layers were washed with brine (10 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. This was concentrated and the residue was purified on a silica column to give the target aryl ether derivative.

**(S,Z)-4-((2-chloropyrimidin-4-yl)oxy)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)pent-2-enamide (20a).** Yield: 4.6 mg (31%) of **20a** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.28 (d, *J* = 5.7 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.66 (d, *J* = 5.7 Hz, 1H), 6.65-6.60 (m, 1H), 6.26 (d, *J* = 15.8, 1H), 6.02 – 5.85 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.45 (t, *J* = 7.2 Hz, 1H), 4.60 (t, *J* = 5.2 Hz, 1H), 4.51 – 4.40 (m, 1H), 4.10 – 3.89 (m, 5H), 2.57 (s, 2H), 2.43 (dd, *J* = 7.3, 5.4 Hz, 2H), 2.04 – 1.85 (m, 2H), 1.75 – 1.69 (m, 3H), 1.58 (s, 2H), 1.51 (d, *J* = 6.4 Hz, 3H), 1.40 (s, 3H), 1.28 (s, 3H), 1.23 – 1.11 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 169.77, 164.66, 160.16, 158.98, 142.96, 135.70, 135.53, 128.06, 125.38, 123.45, 107.02, 102.24, 73.05, 71.66, 70.22, 70.08, 69.45, 55.65, 51.05, 43.87, 42.43, 38.56, 34.14, 31.52, 23.76, 20.04, 12.59; HRMS (ESI) *m/z* Calcd for C<sub>27</sub>H<sub>37</sub>ClN<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 534.2371, found 534.237.

**(S,Z)-4-((6-chloropyrimidin-4-yl)oxy)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)pent-2-enamide (20b).** Yield: 5 mg (33%) of **20b** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.66 (t, *J* = 0.7 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 6.78 (t, *J* = 0.7 Hz, 1H), 6.39 (pd, *J* = 6.4, 1.7 Hz, 1H), 6.23 (d, *J* = 15.7 Hz, 1H), 5.92 – 5.82 (m, 2H), 5.59 (dd, *J* = 15.7, 6.5 Hz, 1H), 5.43 (t, *J* = 7.2 Hz, 1H), 4.60 (t, *J* = 5.3 Hz, 1H), 4.45 (ddd, *J* = 11.8, 6.7, 2.4 Hz, 1H), 4.08 – 3.94 (m, 5H), 2.57 (s, 2H), 2.40 (t, *J* = 6.8 Hz, 2H), 2.08 – 1.85 (m, 2H), 1.70 (d, *J* = 1.3 Hz, 3H), 1.51 (d, *J* = 6.4 Hz, 3H), 1.40 (s, 3H), 1.28 (s, 3H), 1.26-1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 169.44, 165.04, 161.06, 158.40, 140.12, 135.70, 135.45, 128.09, 125.33, 124.62, 108.07, 102.44, 73.04, 71.45, 70.35, 70.19, 69.41, 55.65, 51.05, 43.90, 42.44, 38.55, 34.13, 31.52, 23.75, 20.57, 12.57; HRMS (ESI) *m/z* Calcd for C<sub>27</sub>H<sub>37</sub>ClN<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 534.2371, found 534.2374.

**(S,Z)-4-((5-bromopyrimidin-2-yl)oxy)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)pent-2-enamide (20c).** Yield: 5.5 mg (34%) of **20c** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.60 (s, 2H), 8.12 (d, *J* = 8.3 Hz, 1H), 6.28 – 6.17 (m, 2H), 5.97 – 5.84 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.44 (t, *J* = 7.3 Hz, 1H), 4.60 (t, *J* = 5.4 Hz, 1H), 4.52 – 4.38 (m, 1H), 4.14 – 3.83 (m, 5H), 2.57 (s, 2H), 2.40 (t, *J* = 6.3 Hz, 2H), 2.02 – 1.86 (m, 2H), 1.70 (d, *J* = 1.2 Hz, 3H), 1.53 (d, *J* = 6.4 Hz, 3H), 1.39 (s, 3H), 1.29 (s, 3H), 1.24 – 1.10 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.24, 163.04, 162.60, 159.88, 140.22, 135.74, 135.42, 128.15, 125.27, 124.65, 111.79, 102.47, 73.05, 71.79, 70.42, 70.29, 69.41, 55.64, 51.05, 43.84, 42.43, 38.54, 34.12, 31.52, 23.75, 20.66, 12.62; HRMS (ESI) *m/z* Calcd for C<sub>27</sub>H<sub>37</sub>BrN<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 578.1866, found 578.1843.

**(S,Z)-4-((6-chloro-5-methylpyrimidin-4-yl)oxy)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)pent-2-enamide (20d).** Yield: 5.6 mg (36%) of **20d** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.51 (d, *J* = 1.0 Hz, 1H), 8.28 (d, *J* = 8.2 Hz, 1H), 6.33 (dq, *J* = 8.1, 6.3 Hz, 1H), 6.27 – 6.19 (m, 1H), 5.95 – 5.78 (m, 2H), 5.59 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.42 (t, *J* = 7.4 Hz, 1H), 4.58 (t, *J* = 5.4 Hz, 1H), 4.50 – 4.38 (m, 1H), 4.09 – 3.92 (m, 5H), 2.57 (s, 2H), 2.42 – 2.33 (m, 2H), 2.24 (d, *J* = 0.8 Hz, 3H), 2.02 – 1.81 (m, 2H), 1.68 (d, *J* = 1.4 Hz, 3H), 1.51 (d, *J* = 6.4 Hz, 3H), 1.39 (s, 3H), 1.27 (s, 3H), 1.23 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 167.53, 165.21, 159.89, 154.92, 139.94, 135.70, 135.50, 128.12, 125.42, 124.72, 116.73, 102.49, 73.05, 71.48, 70.40, 70.23, 69.43, 55.66, 51.06, 43.90, 42.45, 38.56, 34.17, 31.53, 23.77, 20.73, 12.57, 11.76; HRMS (ESI) *m/z* Calcd for C<sub>25</sub>H<sub>39</sub>ClN<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 548.2527, found 548.2523.

**(S,Z)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)-4-(pyrimidin-2-yloxy)pent-2-enamide (20e).** Yield: 6 mg (44%) of **20e** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.79 (d, *J* = 8.4 Hz, 1H), 8.59 (d, *J* = 4.8 Hz, 2H), 6.93 (t, *J* = 4.8 Hz, 1H), 6.27 – 6.13 (m, 2H), 5.97 – 5.82 (m, 2H), 5.58 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.42 (t, *J* = 7.3 Hz, 1H), 4.60 (t, *J* = 5.4 Hz, 1H), 4.52 – 4.38 (m, 1H), 4.13 – 3.92 (m, 5H), 2.57 (s, 2H), 2.33 (t, *J* = 6.4 Hz, 2H), 2.03 – 1.85 (m, 2H), 1.66 (d, *J* = 1.3 Hz, 3H), 1.53 (d, *J* = 6.4 Hz, 3H), 1.39 (s, 3H), 1.28 (s, 3H), 1.24 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.60, 164.48, 159.56, 139.53, 135.58, 135.50, 128.13, 125.47, 124.91, 114.97, 102.37, 73.08, 70.91, 70.49, 70.28, 69.46, 55.65, 51.06, 43.88, 42.44, 38.56, 34.15, 31.54, 23.78, 20.95, 12.58; HRMS (ESI) *m/z* Calcd for C<sub>27</sub>H<sub>38</sub>N<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 500.2764, found 500.2761.

**(S,Z)-4-((6-chloro-2-(methylthio)pyrimidin-4-yl)oxy)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)pent-2-enamide (20f).** Yield: 6 mg (36%) of **20f** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 6.78 – 6.66 (m, 1H), 6.56 (d, *J* = 8.5 Hz, 1H), 6.40 (d, *J* = 0.7 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 6.09 – 6.01 (m, 1H), 5.86 – 5.78 (m, 1H), 5.61 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.47 (t, *J* = 7.5 Hz, 1H), 4.59 (t, *J* = 5.2 Hz, 1H), 4.50-4.43 (m, 1H), 4.04 – 3.88 (m, 5H), 2.57 (s, 2H), 2.50 – 2.43 (m, 5H), 2.02 – 1.86 (m, 2H), 1.74 (s, 3H), 1.51 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 1.28 (s, 3H), 1.25 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 172.85, 168.97, 164.50, 160.47, 145.69, 135.78, 135.54, 128.15, 125.34, 122.02, 102.32, 73.08, 71.71, 70.20, 69.48, 55.66, 51.07, 43.78, 42.45, 38.59, 34.15, 31.54, 23.78, 19.96, 14.14, 12.65; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>39</sub>ClN<sub>3</sub>O<sub>6</sub>S (M+H)<sup>+</sup> 580.2248, found 580.224.

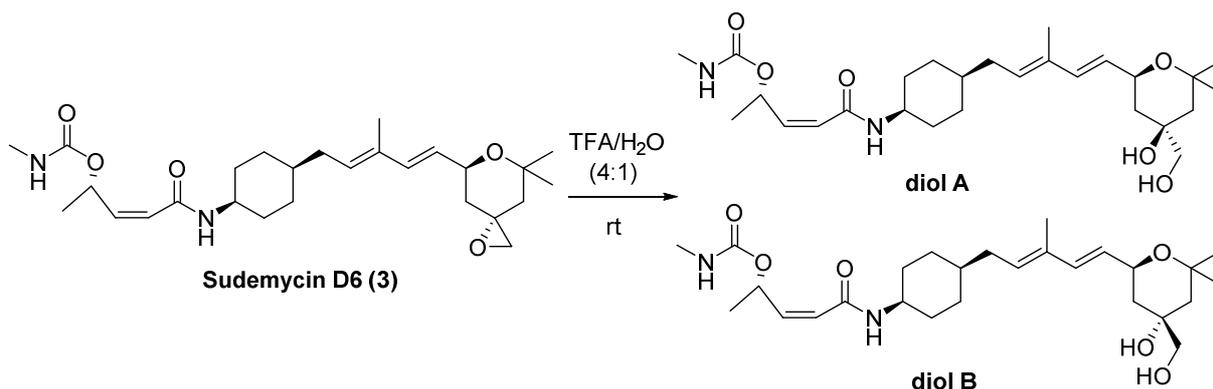
**(S,Z)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)-4-((4-methylpyrimidin-2-yl)oxy)pent-2-enamide (20g).** Yield: 5 mg (34%) of **20g** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.80 (d, *J* = 8.3 Hz, 1H), 8.45 (d, *J* = 5.1 Hz, 1H), 6.79 (d, *J* = 5.1 Hz, 1H), 6.27 – 6.12 (m, 2H), 5.95 – 5.81 (m, 2H), 5.58 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.42 (t, *J* = 7.3 Hz, 1H), 4.59 (t, *J* = 5.4 Hz, 1H), 4.50 – 4.40 (m, 1H), 4.13 – 3.93 (m, 5H), 2.57 (s, 2H), 2.46 (s, 3H), 2.38 – 2.30 (m, 2H), 2.02 – 1.85 (m, 2H), 1.66 (d, *J* = 1.3 Hz, 3H), 1.52 (d, *J* = 6.4 Hz, 3H), 1.40 (s, 3H), 1.28 (s, 3H), 1.24 – 1.12 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 170.86, 165.67, 164.28, 158.21, 139.88, 135.57, 135.54, 128.11, 125.54, 124.69, 114.64, 102.33, 73.08, 70.67, 70.47, 70.22, 69.47, 55.65, 51.07, 43.89, 42.44, 38.56, 34.16, 31.54, 24.17, 23.78, 20.95, 12.56; HRMS (ESI) *m/z* Calcd for C<sub>28</sub>H<sub>40</sub>N<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 514.2917, found 514.2917.

**(S,Z)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)-4-((5-ethylpyrimidin-2-yl)oxy)pent-2-enamide (20h).** Yield: 5 mg (33%) of **20h** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 9.00 (d, *J* = 8.3 Hz, 1H), 8.43 (t, *J* = 0.6 Hz, 2H), 6.22 (dd, *J* = 15.7, 1.0 Hz, 1H), 6.13 – 6.04 (m, 1H), 5.90 – 5.84 (m, 2H), 5.58 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.42 (t, *J* = 7.3 Hz, 1H), 4.60 (t, *J* = 5.5 Hz, 1H), 4.50 – 4.41 (m, 1H), 4.14 – 3.95 (m, 5H), 2.60 – 2.51 (m, 4H), 2.34 (t, *J* = 6.3 Hz, 2H), 2.02 – 1.85 (m, 2H), 1.65 (d, *J* = 1.3 Hz, 3H), 1.52 (d, *J* = 6.4 Hz, 3H), 1.39 (s, 3H), 1.27 (s, 3H), 1.22 (t, *J* = 7.6 Hz, 3H), 1.19 – 1.13 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.73, 163.19, 158.76, 139.36, 135.62, 135.40, 129.83, 128.15, 125.40, 125.01, 102.38, 73.07, 70.63, 70.49, 70.28, 69.43, 55.65, 51.07, 43.90, 42.44, 38.56, 34.17, 31.53, 23.77, 22.69, 21.03, 15.23, 12.55; HRMS (ESI) *m/z* Calcd for C<sub>29</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub> (M+H)<sup>+</sup> 528.3074, found 528.3078.

**(S,Z)-N-((2R,5R)-2-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-yl)-4-((4-methoxypyrimidin-2-yl)oxy)pent-2-enamide (20i).** Yield: 5.2 mg (35%) of **20i** as a viscous oil. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.93 (d, *J* = 8.2 Hz, 1H), 8.29 (d, *J* = 5.8 Hz, 1H), 6.36 (d, *J* = 5.8 Hz, 1H), 6.27 – 6.12 (m, 2H),

5.95 – 5.82 (m, 2H), 5.58 (dd,  $J = 15.7, 6.6$  Hz, 1H), 5.43 (t,  $J = 7.3$  Hz, 1H), 4.59 (t,  $J = 5.4$  Hz, 1H), 4.51 – 4.41 (m, 1H), 4.11 – 3.92 (m, 8H), 2.57 (s, 2H), 2.35 (t,  $J = 6.3$  Hz, 2H), 2.01 – 1.86 (m, 2H), 1.67 (d,  $J = 1.3$  Hz, 3H), 1.52 (d,  $J = 6.4$  Hz, 3H), 1.39 (s, 3H), 1.28 (s, 3H), 1.23 – 1.13 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  171.72, 165.63, 164.35, 158.01, 139.86, 135.58, 135.52, 128.10, 125.55, 124.62, 102.44, 102.35, 73.06, 70.97, 70.45, 70.23, 69.45, 55.65, 53.98, 51.05, 43.89, 42.45, 38.56, 34.15, 31.53, 23.77, 20.93, 12.57; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{28}\text{H}_{40}\text{N}_3\text{O}_7$  ( $\text{M}+\text{H}$ ) $^+$  530.2866, found 530.2874.

#### Scheme S4: Synthesis of Sudemycin D6 diol:



An ice-cooled stirred solution of compound **3** (75 mg, 0.15 mmol) in a mixture of THF and water (1 mL, 4:1) was slowly treated with a solution of trifluoroacetic acid (0.47 mL, 1M in THF). The reaction was allowed to warm to room temp and stirred overnight. UPLC and SFC showed the formation of two products with the correct mass for the diastereomeric diols. Saturated aqueous  $\text{NaHCO}_3$  (4 mL) was added and the product was extracted with EtOAc (3x10 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated under vacuum. The residue was initially purified by silica column (2-10% MeOH in methylene chloride) and gave diol (60 mg) as a mixture of two diastereomers. These two diastereomers were separated by prep-SFC using an OD-H column (25% MeOH) to give diol A (25 mg, 32%) and diol B (20 mg, 26%) as viscous oils. Data for Diol A:  $^1\text{H}$  NMR (400 MHz, Chloroform- $d$ )  $\delta$  8.09 (d,  $J = 8.1$  Hz, 1H), 6.24 (d,  $J = 15.7$  Hz, 1H), 5.81 (d,  $J = 11.1$  Hz, 1H), 5.62 (t,  $J = 10.3$  Hz, 1H), 5.56 – 5.46 (m, 3H), 4.90 (s, 1H), 4.15 – 4.01 (m, 2H), 3.67 (dd,  $J = 56.2, 11.0$  Hz, 2H), 2.77 (d,  $J = 4.2$  Hz, 3H), 2.42 (s, 2H), 2.09 (t,  $J = 7.1$  Hz, 2H), 1.92 (d,  $J = 13.2$  Hz, 1H), 1.80 (d,  $J = 13.8$  Hz, 1H), 1.74 – 1.68 (m, 2H), 1.71 (s, 3H), 1.62 – 1.52 (m, 5H), 1.48 – 1.36 (m, 4H), 1.30 (s, 6H), 1.22 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.31, 157.07, 136.77, 136.09, 133.72, 132.02, 126.81, 125.95, 72.41, 71.18, 69.65, 69.10, 67.80, 45.43, 44.97, 40.57, 36.63, 34.40, 33.20, 29.56, 29.42, 27.62, 27.40, 27.26, 24.99, 20.62, 12.47; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{27}\text{H}_{45}\text{N}_2\text{O}_6$  ( $\text{M}+\text{H}$ ) $^+$  493.3278, found 493.3275.

Data for Diol B:  $^1\text{H}$  NMR (400 MHz, Chloroform- $d$ )  $\delta$  8.03 (d,  $J = 7.6$  Hz, 1H), 6.27 (d,  $J = 15.7$  Hz, 1H), 5.81 (d,  $J = 11.1$  Hz, 1H), 5.66 – 5.52 (m, 3H), 5.48 (t,  $J = 7.6$  Hz, 1H), 4.88 (q,  $J = 4.6$  Hz, 1H), 4.58 – 4.43 (m, 1H), 4.10 (m, 1H), 3.38 (d,  $J = 2.2$  Hz, 2H), 2.77 (d,  $J = 4.8$  Hz, 3H), 2.09 (t,  $J = 7.1$  Hz, 3H), 1.75 – 1.66 (m, 2H), 1.71 (d,  $J = 1.2$  Hz, 3H), 1.69 – 1.61 (m, 2H), 1.60 – 1.52 (m, 5H), 1.46 (s, 3H), 1.38 – 1.28 (m, 4H), 1.30 (d,  $J = 5.7$  Hz, 3H), 1.24 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.28, 157.07, 136.75, 135.80, 133.86, 131.63, 127.34, 125.99, 72.18, 71.89, 71.07, 69.12, 67.41, 45.42, 42.72, 39.62, 36.67, 34.44, 32.82, 29.58, 29.44, 27.60, 27.40, 27.24, 24.57, 20.63, 12.46; HRMS (ESI)  $m/z$  Calcd for  $\text{C}_{27}\text{H}_{45}\text{N}_2\text{O}_6$  ( $\text{M}+\text{H}$ ) $^+$  493.3278, found 493.3274.

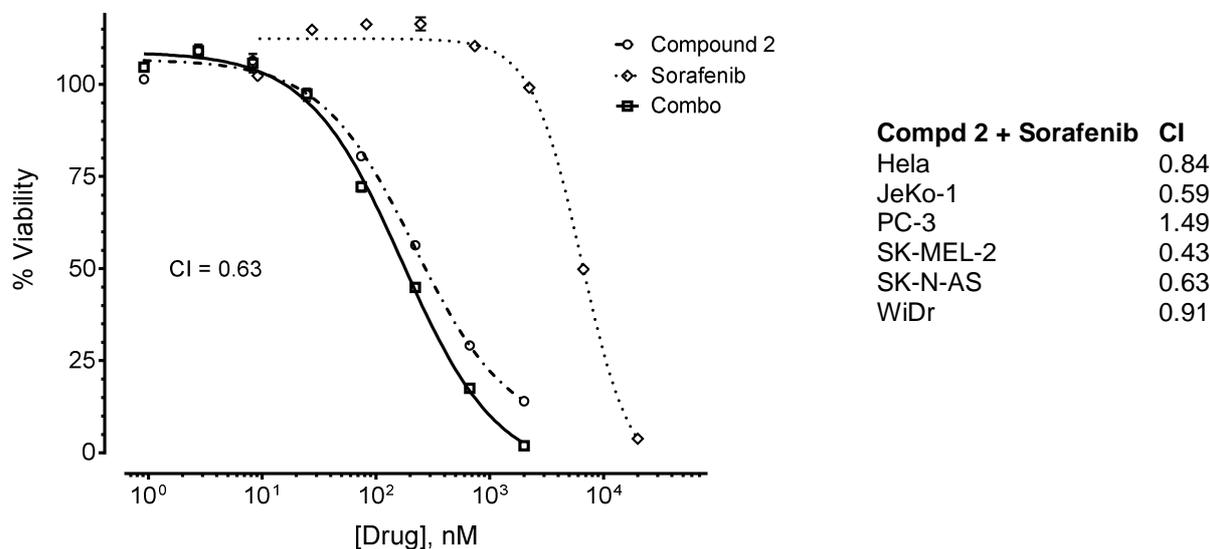
## In Vitro Drug Interaction Assays

Drug	Mechanism	Interaction
5-Fluorouracil	Nucleoside metabolic Inhibitor	Ineffective*
AUDA	Inhibitor of sEH	Synergism
BEZ 235	Dual inhibitor of PI3K and mTOR	Strong synergism
BIBR 1532	Telomerase inhibitor	Ineffective
Bortezomib	Proteasome inhibitor	Antagonism
Camptothecin	DNA topoisomerase I inhibitor	Antagonism
Doxorubicin	Antibiotic inhibitor of DNA topoisomerase II	Ineffective
Etoposide	Topoisomerase II inhibitor	Ineffective
Harringtonine	Inhibitor of protein synthesis	Antagonism
Hydroxyurea	Antimetabolite	Ineffective
Imatinib	Competitive tyrosine kinase inhibitor	Antagonism
Lonidamine	Mitochondrial hexokinase inhibitor	Ineffective
Mitomycin C	DNA cross-linker, alkylating agent	Ineffective
NCND	Inhibitor of sEH	Slightly additive
Nutlin-3	Inhibit the interaction with <i>MDM2</i> -p53	Ineffective
Oxythiamine	Transketolase inhibitor	Ineffective
Panobinostat	Broad-spectrum HDAC inhibitor	Antagonism
PX-866	Irreversible inhibitor of PI3K	Synergism
Silibinin	Inhibitor of P-glycoprotein-mediated cellular efflux & cytochrome P450	Moderate synergism
Sorafenib	Multikinase inhibitor	Synergism
SU 9516	Cyclin-dependent kinase-2 inhibitor	Antagonism
Taxol	Microtubule inhibitor	Antagonism
Temsirolimus	Inhibitor of mTORC1	Synergism

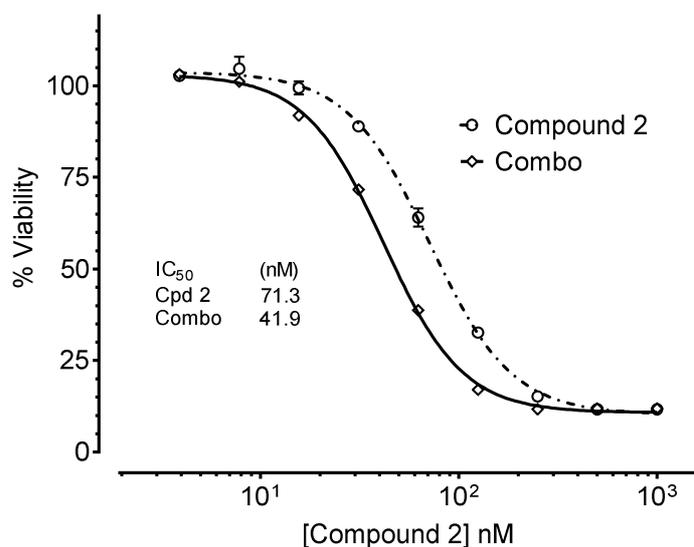
**Supplementary Table S1.** A complete list of the drugs and active agents tested in combination with compounds **1** and **2** along with the observed interaction types. See Supplementary Table 3 below for the ranges of the Combination Index (CI) used. \*Ineffective interaction indicates that the concentration of drug required for the IC<sub>50</sub> is much higher than 1 μM.

Range of CI	Interaction
<0.1	Very strong synergism
0.1–0.3	Strong synergism
0.3–0.7	Synergism
0.7–0.85	Moderate synergism
0.85–0.90	Slight synergism
0.90–1.10	Nearly additive
1.10–1.20	Slight antagonism
1.20–1.45	Moderate antagonism
1.45–3.3	Antagonism
3.3–10	Strong antagonism
>10	Very strong antagonism

**Supplementary Table S2.** Ranges used in CalcuSyn 2.1 to describe the drug interactions in combination studies that were analyzed by the Combination Index (CI) Method.<sup>6</sup>



**Supplementary Figure S2.** The interaction between **2** and Sorafenib is predominantly synergistic. IC<sub>50</sub> curves of drug combinations in SK-NA-S cells are shown on the left, and combination indexes (CI) calculated at the concentration of drugs required to inhibit 50% of cell proliferation (ED<sub>50</sub>) in various tumor cell lines are listed on the right. Each experimental condition was carried out in triplicate; error bars represent the SEM.



**Supplementary Figure S3.** Inhibition of soluble epoxide hydrolase improves the IC<sub>50</sub> of compound **3**. IC<sub>50</sub> curves of drug combinations in HeLa cells are shown. The combination index (CI) cannot be calculated, because AUDA (15 μM) alone is not cytotoxic. The drug doses

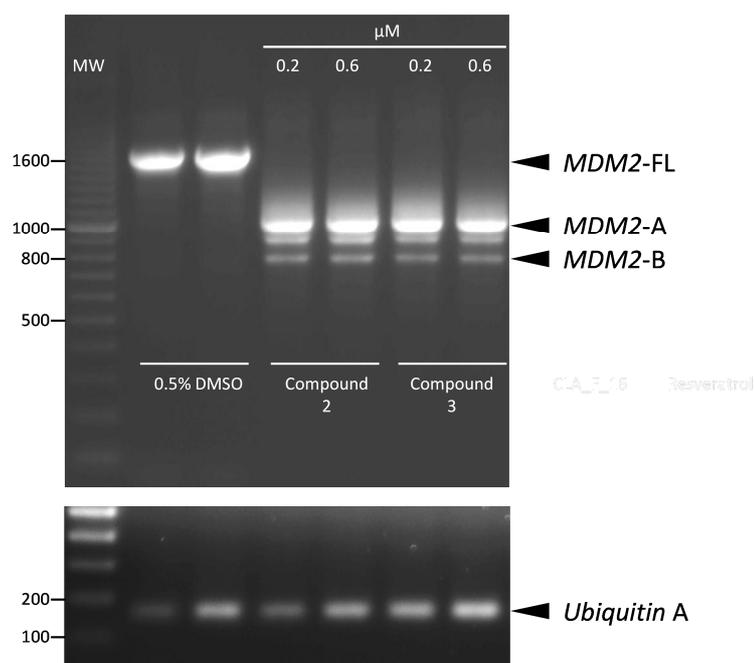
required to inhibit 50% of cell proliferation ( $IC_{50}$ ) were calculated based on the dose-response nonlinear regression 4 Parameter Logistic curve models. Each experimental condition was carried out in quadruplicate; error bars represent the SEM.

Cell Line	CI of 2 + inhibitor	
	PI3K inhibitor (PX-866)	mTOR inhibitor (Temsirrolimus)
HeLa	0.49	0.35
JeKo-1	n.d.	0.37
PC-3	0.67	0.57
SK-MEL-2	0.57	0.68
Sk-N-AS	0.65	0.58
WiDr	0.33	0.44

**Supplementary Table S3.** Combination index (CI) show that interactions between compound 3 and inhibitors of PI3K or mTOR are highly synergistic. n.d.: not determined

### In Vitro Alternative Splicing Assay

In vitro evaluation of *MDM2* alternative-splicing reactions were performed as previously described (ACS Chem. Biol., 2011, 6, 582–589). In short, following an 8 h exposure of exponentially growing cells to drug, total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (1  $\mu$ g) was used to convert the mRNA to cDNA by using random primers, Oligo(dT)<sub>16</sub> and Omniscript Reverse Transcription Kit (Qiagen, Germantown, MD). PCR analyses were performed using specific oligonucleotides (see Supplementary Table S4) and standard amplification protocols. PCR products were separated by agarose gel electrophoresis, visualized by ethidium bromide staining on a UV transilluminator, and recorded. PCR band sizes were calculated by comparing them to the standard EZ Load 100-bp PCR Molecular Ruler (Bio-Rad Laboratories, Hercules, CA). The identities of specific transcripts were verified by sequencing, as previously described (ACS Chem. Biol., 2011, 6, 582–589).



**Supplementary Figure S4.** **2** and **3** induce alternative *MDM2* splicing in Rh18 cells. The upper panel shows the PCR results of the *MDM2* alternative splicing. The lower panel shows the amplification of ubiquitin A, a gene that serves as a loading control because it is an amplified transcript that is not affected by splicing modulators. The cells were exposed to the indicated drug treatments for 8 h.

**Supplementary Table S4.** Oligonucleotides used for assessing mRNA alternative splicing

Gene	Primer	Sequence	Isoform; expected product size (bp)
<i>MDM2</i> (NM_002392)	mdm2-F mdm2-R	CTGGGGAGTCTTGAGGGACC CAGGTTGTCTAAATTCCTAG	<i>MDM2</i> -FL; 1604 <i>MDM2</i> -A; 1019 <i>MDM2</i> -B; 785
<i>Ubiquitin A</i> (NM_001033930)	UbA-F UbA-R	ACCTGACCAGCAGCGTCTGATATT TCGCAGTTGTATTTCTGGGCAAGC	<i>UbA</i> -FL; 165

## In Vivo Pharmacokinetic Studies

### Plasma PK of compound 3 in C.B-17 scid Mice.

#### In Vivo METHODS

**Bioanalysis of compound 3 in C.B-17 scid Mouse Plasma by LC-MS/MS.** We developed a sensitive and specific LC-MS/MS assay for quantitation of compound **3** in plasma for application in a murine pharmacokinetic study. Calibrators and quality controls (QCs) were prepared using 100% methanol stocks in blank C.B-17 scid mouse plasma with lithium heparin anticoagulant. The calibrators and QCs, 25  $\mu$ L each, were stored briefly on wet ice and then protein precipitated with 75  $\mu$ L of ice cold **2** internal standard processing solution. The supernatants, 100  $\mu$ L each, were mixed with 100  $\mu$ L of methanol-10 mM ammonium bicarbonate, pH 7.0 (50:50 v/v) and injected from the 96-well plate to the LC-MS/MS system via a CTC HTS PAL autosampler. Chromatography was performed using a Phenomenex Synergi Fusion-RP C18 column (4.0 $\mu$ m, 50 mm x 2.0 mm) maintained at 35°C, and a linear gradient mobile phase consisting of water:methanol:acetonitrile (22.5:57.5:22.5, v/v) to methanol: acetonitrile (70:30, v/v) and back. Retention times of 0.47 and 0.59 minutes were observed for **3** and **2**, respectively. Detection was performed with an AB Sciex 5500 QTRAP LC-MS/MS System equipped with a Turbo IonSpray® source using the positive multiple-reaction monitoring (MRM) mode. The transitions monitored included m/z 475->288 for **3** and m/z 489->400 for the **2** analog, which served as an internal standard. To conclude, the assay demonstrated adequate sensitivity, precision, and accuracy. The lower limit of quantitation was 1 ng/mL (5X S:N ratio, < 20% CV); intra-run precision and accuracy was  $\leq$  11.2% CV and 96.5 – 103%, respectively. The calibration curve was linear from 1 to 500 ng/mL (R=0.9968) and QC samples above the limit of quantitation (500 ng/mL) were precisely and accurately diluted into the linear range with blank C.B-17 scid mouse plasma.

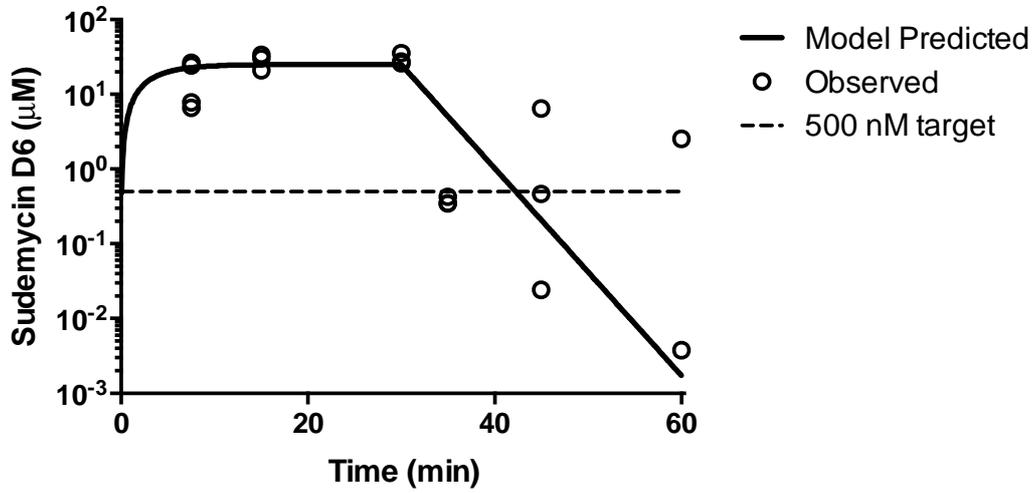
**In Vivo Plasma Pharmacokinetic (PK) Study of compound 3 in C.B-17 scid Mice.** A plasma PK study of **3** in male C.B-17 scid mice receiving a continuous i.v. infusion of **3** (100 mg/kg over 30 minutes) was performed. Compound **3** was formulated in 15% Captisol®/50 mM Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> solution at 3 mg/mL and infused with a syringe pump at a rate of 20  $\mu$ L/min via a jugular vein catheter. Blood samples (1 to 2 samples per mouse, 18 samples from 11 total

mice) were collected by either retro-orbital sinus bleeding or terminal cardiac puncture at the following times after the start of infusion: 7.5, 15, 30 (end of infusion), 35, 45, and 60 minutes. The samples were immediately processed to plasma by centrifugation at 2000g for 5 minutes; the resultant volume of plasma was then protein precipitated with a 3:1 volume of ice cold IS processing solution. The supernatants were immediately placed on dry ice and then stored at -80°C until analysis.

**PK Analyses.** The mouse compound **3** concentration-time data was analyzed using the MLEM population estimation method as implemented in ADAPT5.<sup>7</sup> A linear, one-compartment PK model was fit to the data, and the adequacy of fit was assessed by the -2 log likelihood value, visual predictive checks, and the residual plots. Inter-individual variability in plasma clearance (CL) and volume of distribution (V) was assumed to be log-normally distributed with the covariance matrix constrained to diagonal elements. The residual, intra-individual error was described using an additive and proportional model, with the additive term fixed to the lower limit of quantitation of the LC-MS/MS assay (LLOQ, 1 ng/mL). Beal's M3 method was used to handle data that was below the LLOQ.<sup>8</sup> Other secondary parameters such as the area under the plasma concentration-time curve to infinity ( $AUC_{inf}$ ), maximum plasma concentration ( $C_{max}$ ), and the time of  $C_{max}$  ( $T_{max}$ ) were derived for the population using standard formulae.<sup>9</sup>

The one-compartment model adequately described the mean population compound **3** plasma concentration-time data, with good concordance between observed and population predicted concentrations (Supplementary Figure S5). The mean population PK parameter estimates are listed in Supplementary Table 2. After a 30-minute infusion of **3** at 100 mg/kg in C.B-17 scid mice, the mean CL value of 280 mL/min/kg was considered high as it exceeded hepatic blood flow by 3-fold. The mean V value exceeded total body water (> 0.6 L/kg), suggesting distribution to the peripheral tissues, but was highly variable between mice. The overall PK variability was moderate-to-high, particularly at time points after the end of infusion. The population mean terminal elimination half-life ( $T_{1/2}$ ) was 2.18 minutes, with post-hoc individual estimates ranging from 50 seconds to 12 minutes. The high CL and variability may result from the differential expression of enzymes responsible for compound **3** metabolism. Moreover, both the amount and tissue location of these enzymes and their activity may vary both within and between mice. Despite the rapid CL and PK variability, the target plasma concentration of 500 nM, a concentration associated with tumor spliceosome inhibition in flank xenograft models and in cellular systems, was far exceeded and maintained for an adequate time period.

**Supplementary Figure S5. Plasma Concentration-Time Profile of Intravenous Compound 3 in C.B-17 scid Mice**



**Supplementary Table S5. Population Plasma PK Parameters of Intravenous Compound 3 in C.B-17 scid Mice**

Parameter	Estimate	RSE (%)	IIV (%CV)	RSE (%)
CL (mL/min/kg)	280	28.6	5.21	504
V (L/kg)	0.877	195	113	68.7
T <sub>1/2</sub> (min)	2.18	-	113	-
σ <sub>add</sub> (µM)	0.00211 <sup>a</sup>	-	-	-
σ <sub>prop</sub> (%)	36.4	47.4	-	-
C <sub>max</sub> (µM)	24.1	-	-	-
T <sub>max</sub> (min)	30.0	-	-	-
AUC <sub>inf</sub> (µM-min)	752	-	-	-

<sup>a</sup> Fixed to assay LLOQ (1 ng/mL)

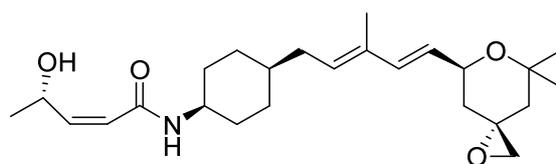
### Animal weight measurements and effects.

The individual mouse weight was measured daily 5 times per week. N= 8 for the vehicle group and 9 for the drug treated group. We found that compound **3** induced weight loss in the animals infused with this splicing modulator. The drug treatment induced a weight loss from the beginning of the drug administration; and the loss of weight continues during the five day infusion period of compound **3**. The average maximum weight loss in the animals treated with compound **3** was 12.7% ( $\pm$  2.3 %, SEM) at day 5 of infusion. The animals in the drug-treated group regain weight immediately after the suspension of drug and return to a normal weight range.

### Compound plasma stability assay and identification of the alcohol metabolite

Compound stability in human and mouse plasma was determined using multiple time points to monitor the rate of disappearance of the parent compound during incubation at 37 deg. C in fresh plasma samples. Sample preparation for plasma stability was modified from Di's et al.<sup>10</sup> A set of incubation times of 0, 15, 30, 60 min, 3, 6, 24, and 48 hours were analyzed. DMSO stock solutions of test compounds were prepared at 10 mM concentration. 1.8  $\mu$ L of each stock was added to 1.8 mL of human or mouse plasma to get the final concentration of 10  $\mu$ M of the test compound. The compound/matrix was mixed and 70  $\mu$ L was transferred to 8 time-point plates (each condition in triplicate). For the Time 0 plate, 140  $\mu$ L of ice-cold acetonitrile containing the internal standard (4  $\mu$ g/ml warfarin) was added to each well and no incubation was performed. For the other seven time-point plates, the plates were incubated at 37 $^{\circ}$ C for indicated times, followed by the quenching of the reaction by the addition of 140  $\mu$ L of ice-cold acetonitrile with internal HPLC standard (4  $\mu$ g/ml warfarin, as a quantification reference) to each well. All plates were tightly sealed and mixed at 600 rpm for 10 min, and then centrifuged at 4000 rpm for 20 min. 120  $\mu$ L of the supernatant were transferred to analytical plates for LC/MS analysis. The LC/MS conditions for Waters ACQUITY-UPLC-MS-UV system used as described separately in the following section. The plasma stability was calculated as the half-life from least-squares fit of a multiple time-point data set, based on a first-order kinetics curve fit.

In the case of ester derivatives such as **18a** and **18b** the UPLC chromatogram (using the system described below) showed a clean proportional increase in a peak with the retention time and mass spectral profile to a sudemycin C alcohol standard, prepared as previously described.<sup>1</sup>



Sudemycin C alcohol

### Analytical UPLC/MS/UV system

LC/MS grade acetonitrile (ACN) was purchased from Fisher Scientific (Loughborough, UK). LC-MS grade methanol, ammonium bicarbonate and formic acid were obtained from Sigma-Aldrich (St. Louis, MO). Milli-Q ultrapure laboratory grade water was used for the aqueous mobile phase. Chromatographic separation was performed in an Acquity UPLC BEH C18 1.7  $\mu$ m, 2.1 x 50 mm column (Waters Corporation, Milford, MA) using an Acquity ultra performance liquid chromatography system. Data was acquired using MassLynx (v. 4.1) and analyzed using the QuanLynx software suite. The LC system was coupled to an SQ mass spectrometer detector. The total flow rate was 1.0 mL/min. The sample injection volume was 10  $\mu$ L. The UPLC column was constantly maintained at 55  $^{\circ}$ C. Samples were separated on the column under acidic or basic mobile phase based on their physiochemical properties. For the acidic mobile phase,

solvent A was 0.1% formic acid in water; and solvent B was 0.1% formic acid in acetonitrile. For the basic mobile phase, solvent A was 10 mM ammonium bicarbonate in water; and solvent B was 100% methanol. Samples were eluted from the column under one of the gradients listed in the table below. The mass spectrometer was operated in positive-ion mode with electrospray ionization. The detector conditions were as follows: capillary voltage 3.4 kV, cone voltage 30 V, source temperature 130 °C, desolvation temperature 400 °C, desolvation gas 800 L/hr, cone gas 100 L/hr. A full scan range from  $m/z = 110-1000$  in 0.2 s was used to acquire MS data. A single ion recording mass spectrometry for each compound was used to determine the quantification of the samples.

UPLC gradient conditions.

Acidic Mobile Phase				Basic Mobile Phase			
Polar Method		Generic Method		Polar Method		Generic Method	
Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B
0	2	0	10	0	2	0	10
0.35	2	0.2	30	0.5	2	0.2	30
1.6	60	1.6	95	1.6	50	1.6	95
1.8	95	1.95	95	1.95	95	1.95	95
1.95	95	2	10	2	2	2	10
2	2						

### Solubility assay<sup>11</sup>

The assay is performed using a Biomek FX ADME-TOX workstation (Beckman Coulter, Inc., Fullerton, CA) to measure compound solubility in a mimicking biological fluid environment. 30  $\mu$ L of 10 mM compound stock solution in DMSO were applied to each well in a stock plate. The compounds were diluted 600 fold in PBS buffer mixed with 1-propanol (1:1, v/v). The concentrations were assessed by UV spectrometry (230-500 nm). The compound solutions were then diluted 100 fold in system solution buffer, incubated at room temperature for 18 h and then filtered through a 96-well filtration plate (pION Inc., Woburn, MA). Fractions were collected from the filtered sample plate; diluted 1:1 (v/v) with 1-propanol and the compound concentration was determined by UV spectrometry. Calculations were carried out by  $\mu$ SOL Evolution software. All tested compounds were assayed in triplicate.

### Rate of Onset Assays (cell proliferation inhibition with short-term drug treatments)

Cells were seeded into 96-well plates in 100  $\mu$ L of cell culture medium (HeLa,  $4 \times 10^4$  cells/well; SK-MEL-2,  $6 \times 10^4$  cells/well). The cells were allowed to attach overnight and the drug treatment was started by adding 50  $\mu$ L of medium containing the splicing modulator to give a final concentration of 1  $\mu$ M in the cell culture medium. Treatment for continuous drug exposure were run in parallel in the same cell culture plate, at concentrations of 0, 0.063, 0.125, 0.25, 0.5 & 1  $\mu$ M for each test compound. DMSO was added to all wells up to 0.5% (v/v) final concentration. For short-term drug treatment conditions, the medium was aspirated at 1, 2, 3 and 4 hours after adding the drug, and then the cells were carefully washed out with PBS to eliminate any residual drug-containing medium. After washing out the drug, 150  $\mu$ L of fresh cell culture medium

containing 0.5% DMSO were added into all washed out wells. The cell viability was determined using CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI) 72 hours subsequent to the addition of the drug. Drug cytotoxicity was calculated as the percentage of cell proliferation inhibition against untreated cells (0.5% DMSO). Eight replications were performed for each experimental condition assayed.

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