Supplemental Data

Chemistry. Chemicals were synthesized according to literature procedures or purchased from Sigma Aldrich (St. Louis, MO 63178, USA), Fluka (St. Louis, MO 63178, USA), Matrix Scientific (Columbia, SC 29224, USA) andnor Thermo Scientific (Florence, KY 41042, USA), unless otherwise noted. Solvents were used from commercial vendors without further purification unless otherwise noted. Nuclear magnetic resonance spectra were determined on a Varian instrument (^{1}H, 400MHz; ^{13}C, 100Mz). LRMS electron-impact (EI) ionization mass spectra were recorded at 70eV on a ThermoFinnigan PolarisQ (ion trap mass spectrometer). Samples were introduced *via* a heatable direct probe inlet. High resolution electron impact (EI) ionization mass spectra were recorded at 25eV on a JEOL JMS-700T MStation (magnetic sector instrument) at a resolution of greater than 10,000. Samples were introduced *via* heatable, direct probe inlet. MALDI mass spectra were obtained on a Bruker Utraflexstreme time-of-flight mass spectrometer (Billerica, MA), using DHB (2,5-dihydroxybenzoic acid) matrix. Purity of compounds was >95% as established by combustion analyses except in for compounds that were viscous liquids that resisted crystallization or were too valuable to sacrifice to combustion. In these cases, purity was established by a combination of high resolution mass spectra and ^{13}C NMR data. Elemental analyses were determined by Atlantic Microlabs, Inc., Norcross, GA. Compounds were chromatographed on preparative layer Merck silica gel F254 or columns using MP Silica 63-200, 60Å, MP EcoChrom (Eschwege, Germany). Organic solutions were dried over anhydrous magnesium sulfate unless otherwise noted.

(1R,2S,3R,4S)- and (1S,2R,3S,4R)-3-(Methyl(phenethyl)carbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (2a, $R^1 = CH_3$; $R^2 = CH_2CH_2C_6H_5$). To a solution of 673 mg (4 mmol) of norcantharidin (**1**) (Alfa Aesar, Ward Hill, MA USA) in 20 mL of anhydrous THF was added 1.08 g (8 mmol, 2 eq) of N-methylphenethylamine in 20 mL of THF was added over a 30 min period at 0°C. The solution was stirred for 17 h and concentrated. The residue was dissolved in 50 mL of ethyl acetate, and washed with 1N HCl solution. The organic layer was dried and filtered. The filtrate is evaporated to dryness to yield white precipitate of **2a**, which proved difficult to purify. Consequently, the methyl ester **2b** was synthesized, purified and hydrolyzed to secure pure **2a**. To 328 mg (1.03 mmol) of **2b** was added 2.5 mL of 28% aqueous ammonia and 2 mL of methanol. The mixture was heated at 55°C in a glass pressure vial for 8 h. The crude product was evaporated to dryness, diluted with 3 mL of water and extracted twice with dichloromethane. The aqueous layer was acidified to pH 1.5 with 2N HCl. The resulting solution was extracted four times with dichloromethane. The extracts were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to yield 56 mg (18%) of **2a** as white amorphous precipitate. The ^1H and ^{13}C NMR spectra of this compound displayed two amide-bond rotamers in a ratio of 1.4 to 1 of the major to minor isomer. The major rotamer displayed the following characteristic NMR signals: ^1H NMR ($CDCl_3$), δ: 2.89 (s, 3H), 3.11 (d, J=5.2 Hz, 1H), 4.54 (d, J=4.8 Hz, 1H), 4.91 (d, J=4.8 Hz, 1H). ^{13}C NMR ($CDCl_3$), δ: 176, 172.4, 139.1, 128.8, 128.7, 126.8, 81.11, 77.7, 52.5, 51.5, 48.6, 36.2, 34.8, 29.8, 26. The minor rotamer displayed the following characteristic NMR signals: ^1H NMR ($CDCl_3$), δ: 2.96 (d, J=5.2 Hz, 1*H*), 2.99 (s, 3*H*), 4.31 (d, J=4.8 Hz, 1*H*), 4.85 (d, J=4.8 Hz, 1*H*). ^{13}C NMR ($CDCl_3$), δ: 175.9, 171.5, 138.1, 128.8, 128.5, 126.4, 80.9,

77.5, 51.4, 51.3, 47.4, 33.5, 34.2, 29.6, 25.8. LRMS, m/e 303 Calcd. for $C_{17}H_{21}NO_4$ 303. HRMS (EI) Calcd. for $C_{17}H_{22}NO_4$ ($M+H^+$): 304.1543. Found: 304.1543.

Methyl (1R,2S,3R,4S)- and (1S,2R,3S,4R)-3-(methyl(phenethyl)carbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylate (2b, $R^1 = CH_3$; $R^2 = CH_2CH_2C_6H_5$). The crude carboxylic acid **2a** was treated with 6 mL of 2N trimethylsilyldiazomethane (12 mmol, 3 eq) in diethyl ether until a light yellow coloration persisted. The solution was stirred for 17 h and concentrated. Crystallization from ethyl acetate led to 735 mg (61%) of **2b**. The ^1H and ^{13}C NMR spectra of this compound displayed two amide-bond rotamers in a ratio of 19 to 1. The major rotamer displayed the following NMR signals: ^1H NMR ($CDCl_3$), δ: 1.02 (dq, J=9.2 and 4 Hz, 1H), 1.34 (dq, J=9.2 and 4 Hz, 1H), 2.76 (d, J=9.6 Hz, 1H), 2.93 (s, 3H), 3.11 (d, J=9.2 Hz, 1H), 3.66 (s,3H), 4.82 (d, J=4.4 Hz, 1H), 4.98 (d, J=4.4 Hz, 1H). Anal. Calcd for $C_{18}H_{23}NO_4$: C, 68.12; H, 7.30; N, 4.41. Found: C, 68.26; H, 7.34; N, 4.40.

2-(4-(2-(4,4'-Dimethoxytrityloxy)ethyl)phenyl)ethanol (11). To 5 g (30 mmol, 1.1 eq) of 1,4-bis(2-hydroxyethyl)benzene (**10**) (Acros Organics, 2440 Geel, Belgium) in 75 mL of anhydrous dichloromethane and 8.4 mL of triethylamine was added 200 mg (1.7 mmol, 0.05 eq) of 4-(N,N-dimethylamino)pyridine followed by 9.24 g (27.3 mmol, 1 eq) of 4,4'-dimethoxytrityl chloride in 25 mL of anhydrous dichloromethane. The latter reagent was added with stirring over 6 h using a syringe pump. The solution was stirred for an additional 22 h at 25°C, diluted with dichloromethane, washed successively with water and brine and dried over anhydrous Na_2SO_4. The crude, light orange oil was flash chromatographed using 3:2 ethyl acetate-hexane with 1% (v/v) trimethylamine to afford 6.64 g (72%) of **11** as a colorless, viscous oil that resisted all efforts to remove traces of

chromatographic solvents: ^{1}H NMR ($CDCl_3$) δ 2.8 (t, J=6.8 Hz, 2), 2.85 (t, J=6.8 Hz, 2), 3.26 (t, J=6.8, 2), 3.74 (s, 3), 3.79 (t, J=6.8 Hz, 2); 6.72-7.38 (m, 17). ^{13}C NMR (DMSO-d_6) δ 37, 55.4 , 65, 86, 113.2, 126.3, 126.8, 127.9, 128.4, 129.5, 130.2, 136.7, 139.7, 145.5. LRMS *m/z* 195 (14), 273 (13), 303 (100), 468 (<1); HRMS (EI) Calcd for $C_{31}H_{32}O_4$: 468.2301. Found: 468.2311.

4-(2-(4,4'-Dimethoxytrityloxy)ethyl)phenethyl p-toluenesulfonate (12). To 2.29 g (12 mmol, 2 eq) of recrystallized p-toluenesulfonyl chloride in 15 mL of anhydrous pyridine at 0^{o}C under an argon atmosphere was added 2.79 g (6 mmol) of **11** in 15 mL of pyridine dropwise over 15 min. The solution was stirred for 17 h at 25^{o}C. The reaction was quenched by stirring with *ca*. 10 g of ice for 10 min. The mixture was diluted with ethyl acetate, and washed successively with saturated copper sulfate solution. The combined copper sulfate solutions were back-extracted with additional ethyl acetate. The combined ethyl acetate solutions were washed with water and dried. The product was chromatographed using 1:2 ethyl acetate-hexane to afford 2.67 g (72%) of **12** as a viscous, glass-like oil that resisted all efforts to remove traces of chromatographic solvents: ^{1}H NMR (DMSO-d_6) δ 2.78 (t, J=6.4 Hz, 2), 2.85 (t, J=6.4 Hz, 2), 3.11 (t, J=7.2 Hz, 2), 3.32 (s, 3), 3.72 (s, 6), 4.2 (t, J=6.4 Hz, 2); 6.8-7. 8 (m, 21); ^{13}C NMR (DMSO-d_6) δ 21.5, 34.4, 36.0, 55.4, 55.5, 64.9, 71.5, 85.8, 113.6, 127, 127.9, 128.1, 128.2, 129.1, 129.5, 130, 130.5, 132.7, 134.8, 136.3, 137.9, 145.2, 145.5, 158.4; LRMS *m/z* 195 (10), 303 (100), 622 (<1); HRMS (EI) Calcd. for $C_{38}H_{38}O_6S$: 622.2389. Found: 622.2366.

4-(2-(4,4'-Dimethoxytrityloxy)ethyl)phenethyl azide (13). To 5.6 g (8.46 mmole, 1 eq) of **12** in 30 mL of hexamethylphosphoramide was added 1.37 g (21.1 mmol, 2.5 eq) of

sodium azide. The mixture was heated at 80°C for 3 h. The mixture was cooled, diluted with ethyl acetate, washed successively with water and brine, and dried. The crude viscous oil (5.81 g) was used in the subsequent step without further purification. Chromatography using 1:2 ethyl acetate-hexane provided a purified sample of **13** as a viscous oil: ^{1}H NMR (DMSO-d_6) δ 2.79 (t, J=6.8 Hz, 2), 2.82 (t, J=7.2 Hz, 2), 3.11 (t, J=6.8 Hz, 2), 3.54 (t, J=7.2 Hz, 2); 3.72 (s, 6), 6.82-7.3 (m, 17); ^{13}C NMR (DMSO-d_6) δ 34.5, 36, 52, 55.4, 55.5, 65, 85.8, 113.52, 113.55, 127, 128.1, 128.2, 129.1, 129.5, 130, 136.3, 136.5, 137.7, 145.5, 158.4; LRMS *m/z* 195 (12), 303 (100), 493 (<1); HRMS (EI) Calcd. for $C_{31}H_{31}N_3O_3$: 493.2365. Found: 493.2364.

O-Ethyl N-(4-(2-(4,4'-Dimethoxytrityloxy)ethyl)phenethyl)urethane (15). To 886 mg (23.3 mmol, 8 eq) of lithium aluminum hydride in 30 mL of anhydrous THF at 0°C under an argon atmosphere was added 5.75 g (11.7 mmol) of **13** in 30 mL of THF. The mixture was stirred for 20 h at 25°C, cooled to 0°C, and quenched by the successive addition of 1.5 mL of water, 1.5 mL of 15% NaOH solution and 4.5 mL of water. The white salts were filtered through a 1 cm Celite cake in a sintered-glass funnel. The Celite was washed with ethyl acetate, and the filtrate was concentrated to afford crude 4-(2-(4,4'-dimethoxytrityloxy)ethyl)phenethyl amine (**14**). Because of O-to-N rearrangements of the dimethoxytrityl group during silica gel chromatography, the crude amine **14** was used directly in the next step. To 4.70 g (11.1 mmole) of the crude amine **14** in 18 mL of anhydrous pyridine at 0°C under an argon atmosphere was added 2.17 mL (27 mmol, 2.4 eq) of ethyl chloroformate dropwise. The solution was stirred for 22 h at 25°C. The mixture was diluted with 2:1 ethyl acetate-hexane, washed with water and brine, and dried. The crude product was flash chromatographed using 1:3 ethyl acetate-hexane

containing 1% (v/v) trimethylamine to afford 3.92 g (86% for two steps) of **15** as a viscous oil: ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J=7.2 Hz, 3), 2.64-2.7 (m, 2), 2.74-2.82 (m, 2), 3.11 (t, J=6.8 Hz, 2), 3.13-3.2 (m, 2); 3.72 (s, 6), 3.95 (q, J=6.8 Hz, 2), 6.82-7.33 (m, 17); ^{13}C NMR (DMSO-d_6) δ 15.1, 35.6, 36, 42.3, 55,4, 55.5, 59.9, 61.2, 65, 67.6, 85.9, 87.1, 113.5, 113.6, 127, 128.1, 128.2, 128.9, 129.4, 129.5, 130, 136.3, 137.2, 137.4, 137.5, 145.5, 156.2, 158.4; LRMS *m/z* 195 (8), 303 (100), 493 (2), 539 (<1); HRMS (EI) Calcd for $C_{34}H_{37}NO_5$: 539.2672. Found: 539.2670. Anal. Calcd for $C_{34}H_{37}NO_5$: C, 75.67; H, 6.91; N, 2.60. Found, C, 75.19; H, 7.05; N, 2.52.

Methyl (1R,2S,3R,4S)- and (1S,2R,3S,4R)-3-((4-(2-(4,4'-dimethoxyltrityloxyethyl)phenethyl)(methyl)carbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylate (17). To 0.98 g (26 mmol, 4 eq) of lithium aluminum hydride in 25 mL of anhydrous THF at 25^{o}C under an argon atmosphere was slowly added 3.51 g (6.51 mmol) of urethane **15** in 25 mL of THF. Foaming was noted during the addition. After foaming subsided, the mixture was refluxed for 20 h, cooled to 0^{o}C, and quenched by the successive addition of 0.4 mL of water, 0.8 mL of 15% NaOH solution, and 1.2 mL of water. The mixture of white salts was filtered through a Celite cake in a sintered glass funnel. The Celite was washed with ethyl acetate, and the filtrate was concentrated to afford 3.13 g of crude 2-(4-(2-(4,4'-dimethoxytrityloxy)ethyl)-phenyl)-N-methylethylamine (**16**) as an oil. Because of the O-to-N rearrangement of the DMT group during silica gel chromatography, the crude amine **16** was used directly in the next steps. To 2.85 g (6.6mmol, 1 eq) of **16** in 25 mL of anhydrous toluene under an argon atmosphere was added a solution of 1.15 g (6.83 mmol, 1.05 eq) of norcantharidin (**1**) (Alfa Aesar, Ward Hill, MA USA) in 6 mL of anhydrous toluene. The solution was

stirred at 25°C for 17 h and concentrated *in vacuo* at a temperature less than 36°C to afford an intermediate carboxylic acid. Anal. Calcd for $C_{19}H_{25}NO_5$: C, 65.90; H, 7.25; N, 4.03. Found, C, 65.90; H, 7.51; N, 3.93. To this carboxylic acid in 20 mL of anhydrous ethyl ether and 10 mL of methanol at 0°C was added dropwise 6.85 mL of 2N trimethylsilyldiazomethane in ethyl ether until light yellow color persisted. The mixture was stirred at 25°C for 12 h, concentrated, and chromatographed using 8:1 ethyl acetate-hexane containing 1% (v/v) of trimethylamine to afford 2.01 g (47% for three steps) of **17** as a foam and as a mixture of rotamers in a 1.1 to 1 ratio. The major rotamer displayed the following characteristic NMR signals: ^{1}H NMR ($CDCl_3$) δ 2.44 (d, J=9.6 Hz, 1H), 2.81 (d, J=8.8Hz, 1H), 2.93 (s, 3H), 3.6 (s,3H), 4.82 (d, J=4.4 Hz, 1H), 4.97 (d, J=4.4 Hz, 1H), 6.78 (d, J=9.2Hz, 4H), 7.36 (t, J=8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.7, 29.1, 33.1, 33.6, 36.2, 49.2, 50.6, 51.9, 52.9, 55.2, 64.7, 77.3, 78.5, 86.9, 112.9, 126.5, 127.7, 128.1, 128.7, 129.3, 129.9, 136.1, 136.5, 137.3, 145.1, 158.3, 170, 171.3. The minor rotamer displayed the following characteristic NMR signals: ^{1}H NMR ($CDCl_3$) δ 2.74 (d, J=9.2Hz, 1H), 3.09 (d, J=9.2Hz, 1H), 2.92 (s, 3H), 3.65 (s, 3H), 4.44 (d, J=5.2 Hz, 1H), 4.83 (d, J=5.2Hz, 1H), 6.79 (d, J=9.2 Hz, 4H), 7.36 (t, J=8.4 Hz, 1*H*). ^{13}C NMR ($CDCl_3$) δ 28.9, 29.3, 34.2, 36.2, 36.3, 50.2, 51.8, 52, 53, 55.2, 64.7, 77.8, 78.8, 86, 113, 126.6, 127.7, 128.1, 128.7, 129.98, 129.96, 136.4, 137, 138, 145.2, 158.3, 170.6, 171.5. HRMS (EI) Calcd. for $C_{41}H_{46}NO_7$ ($M+H^+$): 664.3269. Found: 664.3269.

"Linker" or 2-(4-(2-(Methylamino)ethyl)phenyl)ethan-1-ol. To a solution of 100 mg (0.21 mmol) **16** in 3 mL of methanol was added 38 µL of trifluoroacetic acid at 21°C. The reaction was quenched after 30 min by the addition of 210 mg of solid sodium bicarbonate. The mixture was filtered and concentrated and chromatographed using 1:10

methanol-dichloromethane with 1% (v/v) of triethyamine to afford 29 mg (77%) of 2-(4-(2-(Methylamino)ethyl)phenyl)ethan-1-ol as a viscous oil that resisted efforts to remove traces of solvent: ^{1}H NMR (CH_3OH-d_4) δ 2.44 (s, 3H), 2.79 (t, J=6.4Hz, 2H), 2.80-2.88 (m, 4H), 3.73 (t, J=7.2Hz, 2H), 7.14 (d, J=7.8Hz, 2H), 7.17 (d, J=7.8Hz, 2H). ^{13}C NMR (CH_3OH-d_4) δ 35.6, 35.7, 40, 53.7, 64.4, 129.8, 130.5, 138.1, 138.7.

Methyl (1R,2S,3R,4S)- and (1S,2R,3S,4R)-3-(4-(2-Hydroxyethyl)phenethyl)(methyl)carbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylate (18). To a solution of 873 mg (1.32 mmol) of **17** in 12 mL of methanol was added 50 μL of trifluoroacetic acid at 25^{o}C. The reaction was quenched after 30 min by the addition of 300 mg of solid sodium bicarbonate. The mixture was filtered and concentrated and chromatographed using 1:1:6 methanol-hexane-ethyl acetate to afford 456 mg (96%) of **18** as a colorless foam and as a mixture of rotamers in a 1.2 to1 ratio. The major rotamer displayed the following characteristic NMR signals: ^{1}H NMR ($CDCl_3$) δ 2.54 (d, J=9.6Hz, 1H), 2.91 (d, J=9.6Hz, 1H), 2.95 (s, 3H), 3.66 (3H), 4.72 (d, J=4Hz, 1H), 4.98 (d, J=4Hz, 1H). ^{13}C NMR ($CDCl_3$) δ: 28.9, 29.2, 33.1, 34.3, 39, 49.4, 50.4, 52, 52.8, 63.6, 77.4, 78.8, 129.1, 129.2, 136.4, 137.2, 170.3, 171.6. The minor rotamer displayed the following characteristic NMR signals: ^{1}H NMR ($CDCl_3$) δ 2.73 (d, J=9.6Hz, 1H), 3.11 (d, J=9.6Hz, 1H), 2.95 (s, 3H), 3.63 (s, 3H), 4.43 (d, J=5.2Hz, 1H), 4.87 (d, J=5.2Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 29.2, 29.4, 33.7, 36.2, 38.9, 50.3, 51.9, 52.1, 53.1, 63.7, 77.9, 78.9, 129.1, 129.6, 136.8, 137.6, 170.8, 171.6. HRMS (EI) Calcd. for $C_{19}H_{26}NO_5$ ($M+H^+$): 362.1962. Found: 362. 1955.

Bisammonium (1S,2R,3S,4R)- and (1R,2S,3R,4S)-3-((4-(2-(((((2R,3R,4R,5R)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydro-furan-3-yl)oxy)oxidophosphoryl)oxy)ethyl)phenethyl)(methyl)carbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylate (**20**)**.** To 309 mg (0.823 mmol, 1 eq) of **18** and 50 mg (0.432 mmol, 0.5 eq) of 4,5-dicyanoimidazole under an argon atmosphere was added a solution of 752 mg (0.864 mmol, 1.05 eq) of 5'-*O*-(4,4'-dimethoxytrityl)-N^2-isobutyryl-2'-*O*-methylguanosine-3'-*O*-(2-cyanoethyl)-phosphoramidite in 7.5 mL of anhydrous acetonitrile at 25°C over a 10 min period. The mixture was stirred at 25°C for 12 h at which time the solution became clear. The solution was concentrated to afford a white solid **19** that was used directly in the next step. To 0.823 mmol of crude **19** in 17 mL of 1:2:7 mixture of pyridine-water-THF at 0°C was added 241 mg (0.949 mmol) of iodine in 17 mL of 1:2:7 mixture of pyridine-water-THF. The mixture was concentrated and chromatographed using 1:20 methanol-dichloromethane to afford 507 mg (58%) of an intermediate, protected phosphate: HRMS (pEI) Calcd. for $C_{58}H_{69}O_{15}N_7P$ (M+H): 1146.4584. Found: 1146.4589. To 258 mg (0.225 mmol) of this phosphate in 4 mL of methanol was added 10 μL of trifluoroacetic acid at 25°C, and after 30 min, the reaction was quenched by the addition of 100 mg of solid sodium bicarbonate. The mixture was filtered, concentrated and chromatographed using 3:20 methanol-dichloromethane to yield 166 mg (88%) of the DMT-deprotected phosphate as a white solid: HRMS (pEI) Calcd for $C_{38}H_{50}O_{13}N_7P$ (M+H): 844.3277. Found: 844.3276. To 55.6 mg (0.0659 mmol, 1 eq) of the DMT-deprotected phosphate in a 5 mL thick-walled glass, screw-cap vial equipped with a magnetic stirrer was added 3 mL of 28% ammonium hydroxide (20.8 mmol, *ca*. 300 eq). The mixture was heated at 66°C for 5.5 h at which time the

solution became clear. The mixture was evaporated to dryness under vacuum at a temperature less than 36^{o}C. The residue was diluted with 10 mL of anhydrous acetonitrile, stirred and heated at 60^{o}C for 1 h and cooled to afford a precipitate that was collected on a sintered, glass filter, washed with 1 mL of cold, anhydrous acetonitrile to remove traces of acetonitrile-soluble by-products, and dried under high vacuum at ambient temperature for 24 h to yield 29 mg (60%) of **20** as a white precipitate. FTMS-nESI between 200 and 1200 *m/z*: 352.11 (100%) $[(M-2H^{+})^{2-}]/2$, 705.2276 (45%) $(M-H^{+})^{-}$ and HRMS (pESI) Calcd. for $C_{30}H_{40}O_{12}N_6P$ $(M+H^{+})^{+}$: 707.2442. Found: 707.2440 (0.114ppm); HRMS (nEI) Calcd. for $[(C_{30}H_{37}O_{12}N_6P)(NH_4)_2\text{-}2H^{+}]$ for doubly charged negative ion: 352.1104. Found: 352.1100 (0.71ppm).

Biology Methods. RT-PCR. For reverse transcription, 400 ng of total RNA (200 ng/µl), 5 pmol of reverse primer, and 40 units of SuperScript II reverse transcriptase were mixed in 5 µL of RT buffer: 50mM Tris-HCl, pH8.3; 75mM KCl; 3mM MgCl2; 10mM DTT; 0.5mM dNTPs; 1mM reverse primer SMNex8rev; and 40U of SuperScript II RT (Life Technologies). To reverse transcribe the RNA, the reaction was incubated at 42 °C for 50 min and followed by 70°C for 15 min. 2 µL of the RT reaction was used for cDNA amplification. The reaction was performed in 20 µL and contained 0.2 µM of specific forward and reverse primers, 200 µm dNTPs, 1× *Taq* polymerase buffer, 1.5mM $MgCl_2$ and 2 unit of Platinum *Taq* DNA polymerase (Life technologies). The amplification was carried out in an Eppendorf PCR system thermocycler under the following conditions: initial denaturation for 4 min at 94 °C; 28 cycles of 30 s at 94 °C, 30 s at 60 °C; and extension of 45 s at 72 °C. After the last cycle, the reaction was held for 5 min at the extension temperature to complete the amplification of all products. Reverse

transcription was performed using SMNex8rev (GCCTCACCACCGTGCTGG). PCR was performed using pCI (GGTGTCCACTCCCAGTTCAA) and SMNex8rev (GCCTCACCACCGTGCTGG).

Treatment of HEK293 cells with compound 20. Exponentially growing HEK293 cells were split at a density of about 3 x 10^5 cells/10 cm^2. Twenty-four hours later, the cells were transfected with 0.5µg SMN2 minigene and then treated with compound **20** at appropriate concentration for the indicated times. After an additional 14-16 h, total RNAs were extracted from transfected and treated HEK293 cells.