SUPPLEMENTAL INFORMATION:

Synthesis and characterization of diazirine alkyne probes for the study of intracellular cholesterol trafficking

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Supplemental Table S1: monitored ion transitions

Analyte	Parent Ion	Product Ion(s)
d4-cholesteryl oleate (internal standard)	672.635	369.400
d7-cholesteryl oleate	675.679	376.400
d16-cholesteryl oleate	684.735	376.400
d17-LKM38 oleate (internal standard)	723.600	407.300
LKM38 oleate	706.600	407.300
d9-LKM38 oleate	715.600	407.300
d5-cholestenone (internal standard)	530.600	126.100, 389.460
d7-cholestenone	532.600	126.100, 391.480
LKM38-one	563.600	126.100, 422.400

Supplemental Table S2: Immunoprecipitation and western blotting conditions

Target	**Antibody**	**IP**	**WB**
NPC1	Millard et al., JBC 2000, 275:38445	1.5 μg /50 μL beads Overnight incubation	1:2000 in 1% BSA TBS-T
NPC2-HA	Abcam Ab9110	1.5 μg/50 μL beads Overnight incubation	1:7000 in 1% BSA TBS-T
Caveolin	Abcam Ab2910	4 μg/50 μL beads, BS^3 crosslinked 3 hour incubation	1:4000 in 5% BSA TBS-T
VAMP7	Bethyl-Laboratories A304-344A	4 μg/50 μL beads 3 hour incubation	1:5000 in 5% milk TBS-T
GAPDH	Abcam Ab2302	Not applicable	1:10000 in 5% milk TBS-T

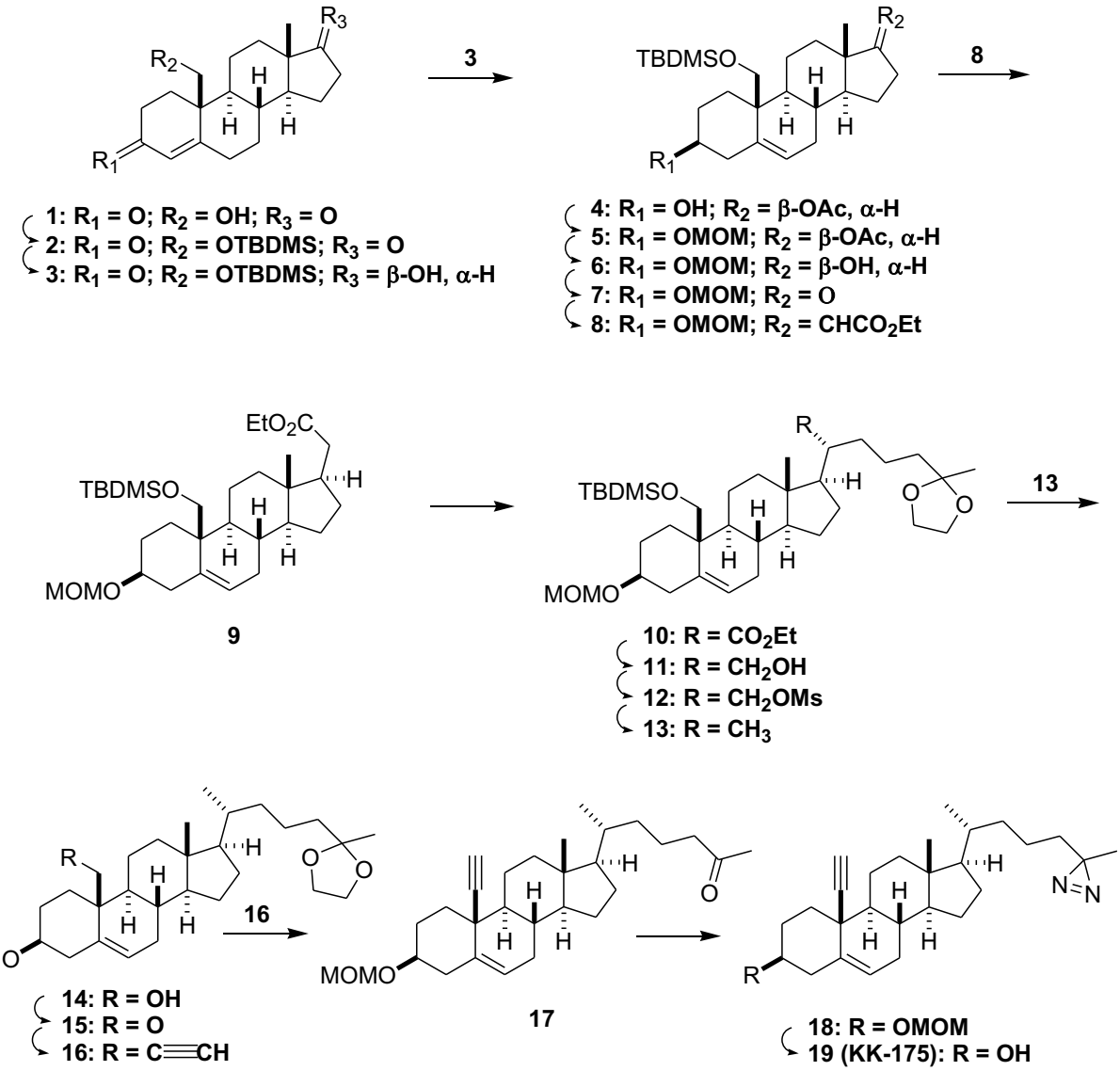
Beads: Dynabeads™ Protein A (Novex)

TBS-T: 140 mM NaCl, 3 mM KCl, 25 mM Tris Base, 0.05% Tween-20, pH 7.4

Secondary incubations were performed at 1:10,000 using anti-Rabbit-HRP (Jackson Immuno) or anti-Chicken-HRP (Millipore Sigma).

SUPPLEMENTAL METHODS

Synthesis of KK-175



(17β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-17-hydroxy-androst-5-en-3-one (3). To a stirred cold (10 °C) solution of steroid **2** (12.5 g, 30 mmol) in EtOH (100 mL) and THF (10 mL) was added dropwise a solution of $NaBH_4$ (304 mg, 8 mmol, 0.27 eq) in EtOH (20 mL). The reaction was slowly warmed to –5 °C and then to 0 °C while the reaction was monitored by TLC. After 4 h, the reaction was acidified by adding saturated aqueous NH_4Cl. The EtOH and THF were removed under reduced pressure to give a residue. Water was added and the product was extracted into CH_2Cl_2. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 10-20% EtOAc in hexane) to yield steroid **3** as a solid (8.7g, 69%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.84 (s, 1H), 3.87 (dd, J = 11. 2 Hz, J = 9.8 Hz, 2H), 3.61 (t,

J = 6.4Hz, 1H), 0.83 (s, 9H), 0.77 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 200.2, 178.1, 125.8, 81.4, 65.7, 54.1,50.9, 43.6, 42.8, 36.8, 36.3, 34.8, 33.6, 33.3, 31.5, 30.3, 25.7, 23.2, 21.2, 17.9, 11.2, –5.8, –5.9.

(3β,17β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-androst-5-ene-3,17 diol, 17-acetate (4). To a cold (0 °C) stirred mixture of steroid 3 (4.5 g, 11 mmol), NaI (5 g, 33 mmol) and acetic anhydride (25 mL) was added trimethylchlorosilane (4 ml, 32 mmol) and the reaction was warmed to room temperature and stirred for 15 h. The reaction was diluted with hexane (250 mL) and stirred. The hexane layer was decanted and added to a cold solution of triethylamine (15 mL):hexane (200 mL) and stirred for 10 min. To the cold solution was added aqueous $NaHCO_3$ solution (200 mL) and stirring was continued for 40 min. The biphasic mixture was transferred to a separatory funnel and the hexane layer was separated. The aqueous solution was extracted with EtOAc (2 x 60 mL) and the combined organic extracts were dried over Na_2SO_4 and the solvents removed under reduced pressure to give an oil. The crude product was immediately purified by flash column chromatography to give the intermediate 3,5-diene,-3,17-diacetate (5.0 g, 90%), which was immediately converted to steroid **4**.

To the 3,5-diene-3,17diacetate (5.0 g, 9.9 mmol) in EtOH (50 mL) and THF (5 mL) was added $NaBH_4$ (950 mg, 25 mmol) in portions over a period of 30 min and the reaction was allowed to stir at room temperature for 17 h. The reaction was acidified by adding saturated aqueous NH_4Cl and the product was extracted into CH_2Cl_2. The CH_2Cl_2 was washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to give a viscous oil. The crude product was purified by flash column chromatography (silica gel eluted with 15-25% EtOH in hexane) to give steroid **4** (3.25 g, 65%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.40 (b s, 1H), 4.58 (t, *J* = 8.2 Hz, 1H), 3.74 (d, *J* = 10.5 Hz, 1H) 3.57 (d, *J* = 10.5 Hz, 1H), 3.54 (m, 1H), 2.03 (s, 3H), 0.87 (s, 9H), 0.83 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 171.2, 136.7, 124.7, 82.8, 71.4, 63.1, 51.6, 50.4, 42.6, 42.5, 41.3, 37.1, 32.7, 32.5, 31.9, 31.1, 27.5, 25.8, 23.5, 21.3, 21.1, 18.1, 12.20, –5.63, –5.66.

(3β,17β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-androst-5-en-17 ol, 17-acetate (5). To a cold solution (0 °C) of steroid **4** (4.63 g, 10 mmol), Hunig's base (5.2 ml, 30 mmol) and CH_2Cl_2 (40 mL) was added chloromethyl methyl ether (1.52 ml, 20 mmol) and the reaction was warmed to room temperature and stirred for 12 h. The reaction was quenched by adding saturated aqueous $NaHCO_3$ solution and the product extracted into CH_2Cl_2. The CH_2Cl_2 was washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The crude product

was purified by flash column chromatography (silica gel eluted with 10-15% EtOAc in hexane to give steroid **5** (4.86g, 96%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.55 (b s, 1H), 4.69 (s, 2H), 4.59 (t, *J* = 8.2Hz, 1H), 3.75 (d, *J* = 10.6 Hz, 1H), 3.59 (d, *J* = 10.9 Hz, 1H), 3.46 (m, 1H), 3.37 (s, 3H), 2.04 (s, 3H), 0.88 (s, 9H), 0.84 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 171.3, 136.6, 124.9, 94.7, 82.8, 76.6, 63.1, 55.2, 51.6, 50.4, 42.6, 41.5, 39.9, 37.1, 32.7, 32.5, 31.11, 29.3, 27.6, 25.8, 23.5, 21.3, 21.2, 18.1, 12.2, –5.61, –5.64.

(3β,17β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-androst-5-en-17 ol (6). A mixture of steroid **5** (4.05 g, 8 mmol), K_2CO_3 (1.28g, 10 mmol) and MeOH (25 ml) was heated at reflux for 2h. The reaction mixture was cooled and the MeOH was removed under reduced pressure to give a solid. Water (200 mL) was added and the product was extracted into CH_2Cl_2. The CH_2Cl_2 was washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 20-35% EtOAc in hexane) to give steroid **6** (3.6 g, 97%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.55 (b s, 1H), 4.69 (s, 2H), 3.76 (d, *J* = 10.4 Hz, 1H), 3.61 (m, 2H), 3.46 (m, 1H), 3.37 (s, 3H), 0.88 (s, 9H), 0.78 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.6, 124.9, 94.6, 81.7, 76.6, 63.1, 55.2, 51.9, 50.6, 42.9, 41.5, 39.9, 36.9, 32.7, 32.7, 31.1, 30.4, 29.3, 25.8, 23.4, 21.4, 18.1, 11.2, –5.62 (2 X C).

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-androst-5-en-17 one (7). To a stirred solution of steroid **6** (3.25 g, 7 mmol) in CH_2Cl_2 (25 mL), was added pyridinium chlorochromate (3.02 g, 14 mmol) in portions and the reaction was stirred for 6 h. The reaction mixture was diluted with hexane (150 mL) and the supernatant solution was passed through a silica gel column eluted with hexane and then by 10-20% EtOAc in hexane. After solvent removal under reduced pressure, steroid **7** (2.95 g, 91%) was obtained as a colorless solid which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.60 (b s, 1H), 4.69 (s, 2H), 3.80 (d, *J* = 11 Hz, 1H), 3.61 (d, *J* = 11 Hz, 1H), 3.46 (m, 1H), 3.38 (s, 3H), 0.92 (s, 3H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 221.4, 136.7, 124.6, 94.7, 76.5, 63.3, 55.2, 52.4, 50.6, 47.8, 41.6, 39.9, 35.8, 33.0, 32.4, 31.8, 30.4, 29.3, 25.8 (3 X C), 21.8, 21.0, 18.2, 14.2, 13.9, –5.57 (2 X C).

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-pregna-5,17(20)-dien-21-oic acid, ethyl ester (8). To a warm (40 °C) mixture of steroid **7** (2.8 g, 6 mmol), triethyl phosphonoacetate (5.9 mL, 30 mmol) in EtOH (100 mL) was added a solution of sodium ethoxide in EtOH (690 mg sodium in 12 mL of ethanol, 30 mmol) dropwise over a

period of 10 min. The reaction mixture was heated at reflux for 18 h. The reaction mixture was cooled and the ethanol was removed under reduced pressure and the residue was cooled in an ice-water bath and neutralized by adding aqueous 6N HCl. The mixture was further diluted with water (150 mL) and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to give an off-white solid. The crude product was purified by flash column chromatography (silica gel eluted with 10-20% EtOAc in hexane to give steroid **8** as a white solid (3.0 g, 95%) which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (b s, 1H, m), 5.54 (t, *J* = 2.2 Hz, 1H), 4.69 (s, 2H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.78 (d, *J* = 10.6 Hz, 1H), 3.61 (d, *J* = 10.6 Hz, 1H), 3.43 (m, 1H), 3.38 (s, 3H), 2.82 (m, 2H), 1.28 (t, *J* = 7.0 Hz, 3H), 0.87 (s, 9H), 0.87 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 167.5, 136.6, 124.9, 108.4, 94.7, 76.6, 63.2, 59.5, 55.2, 54.5, 50.6, 46.3, 41.6, 39.9, 35.5, 32.8, 32.4, 31.3, 30.4, 29.3, 25.8 (3 X C), 24.40, 21.65, 18.52, 18.15, 14.38, –5.59, –5.60.

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-pregna-5-en-21-oic acid, ethyl ester (9). A mixture of steroid **8** (2.66 g, 5 mmol), 5% Pt-C (70 mg) in EtOH (100 mL) was hydrogenated in a Parr hydrogenation apparatus at 20 psi H_2. The reaction was stopped every half-hour and checked by TLC for the disappearance of steroid **8** by loss of its UV on the TLC plate. The reaction was run for about 3 h. The crude reaction mixture was loaded on a silica gel column and eluted with 40% EtOAc in hexane to give an inseparable mixture of steroid **9** containing *ca.*10% of a steroid in which the Δ^5-double bond had also been reduced (2.6 g). The following bromination, chromatography, debromination sequence was used to separate the two steroid products.

To crude a mixture of crude steroid product **9** (2.6 g, 4.9 mmol) and NaOAc (1.64 g, 20 mmol) in diethyl ether (100 mL) was added dropwise bromine (0. 36 mL, 7 mmol) dissolved in acetic acid (8 mL) and the reaction was stirred for 20 min. The reaction was quenched with 5% aqueous sodium thiosulfate solution (20 mL) followed by careful addition of aqueous saturated $NaHCO_3$ (100 mL). After stirring for 10 min, the biphasic reaction mixture was transferred to a separatory funnel and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to give an oil. Flash column chromatography (silica gel eluted with 5-20% EtOAc in hexane) yielded two dibromides along with the readily removed non-brominated steroid in which the Δ^5-double bond had been reduced. The less polar 5α,6β-dibromide was obtained as an oil (1.73 g, 50%) which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.80 (b s, 1H), 4.71 (s, 2H), 4.36 (m, 1H), 4.31 (d, *J* = 10.6 Hz, 1H), 4.12 (q, *J* = 7 Hz, 1H), 3.97 (d, *J* = 11 Hz, 1H), 3.39 (s, 3H), 1.26 (t, *J* = 7 Hz, 3H), 0.90 (s, 9H), 0.66 (s, 3H), 0.10 (s, 3H), 0.09 (s, 3H).

The more polar 5β,6α-dibromide was obtained as an oil (1.04 g, 30%) which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56 (m, 1H), 4.78 (d, *J* = 7 Hz, 1H), 4.63 (d, *J* = 7 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.04 (b s, 1H), 3.95 (d, *J* = 11.4 Hz, 1H), 3.78 (d, *J* = 7 Hz, 1H), 3.41 (s, 3H), 2.86 (d, J = 16.4 Hz, 1H), 1.26 (t, *J* = 7 Hz, 1H), 0.93 (s, 9H), 0.64 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H).

The two bromides were combined (2.77 g, 4 mmol), dissolved in acetic acid (10 mL) and EtOAc (30 mL), zinc dust (1.3 g, 20 mmol) was added and the reaction was vigorously stirred at room temperature for 2 h. The reaction was filtered through a funnel fitted with cotton plug, and the filter-cake was washed with EtOAc. The combined filtrate and washings were stirred in a round-bottom flask and the acetic acid was carefully neutralized by adding saturated aqueous $NaHCO_3$. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an off-white solid. Purification by flash column chromatography (silica gel eluted with 10-25% EtOAc in hexane) yielded steroid **9** (2.0 g, 95%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.56 (b s, 1H), 4.69 (s, 2H), 4.12 (q, *J* = 7.5 Hz, 2H), 3.75 (d, *J* = 10.6 Hz, 1H), 3.59 (d, *J* = 10.9 Hz, 1H), 3.46 (m, 1H), 3,38 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H), 0.87 (s, 9H), 0.65 (s, 3H), 0.3 (s, 3H), 0.02 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 174.0, 136.6, 125.1, 94.6, 76.7, 63.1, 60.1, 56.2, 55.2, 50.7, 46.8, 42.1, 41.6, 39.9, 37.6, 35.3, 32.7, 32.7, 31.6, 29.7, 29.3, 28.2, 25.8, 24.5, 21.5, 18.1, 14.2, 12.7, –5.6, –5.63.

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-27-norcholest-5-en-25-oxo-21-oic acid, ethyl ester, cyclic 1,2-ethanediyl acetal (10). To THF (15 mL) containing 1 M LDA in THF (6 ml, 6 mmol) at –78 °C was added steroid **9** (1.1 g, 2.1 mmol) dissolved in THF (5 mL) and the reaction was stirred at -78 °C for 1 h. To the HMPA (1 mL) was then added and the reaction darkened in color. Stirring was continued at -78 °C for 10 minutes. To that solution was added 2-(3-iodopropyl)-2-methyl-[1,3]dioxolane (2.04 g, 8 mmol) in THF (4 mL) and the reaction was stirred at -78 °C for 1 h. The reaction was slowly allowed to warm to room temperature and stirring at room temperature was continued for 12 h. The reaction was quenched by adding saturated aqueous NH_4Cl solution and the product was extracted into EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give a solid. The crude product was purified by flash column chromatography (silica gel eluted with 10-20% EtOAc in hexane to give steroid **10** (1.25 g, 90%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.54 (b s, 1H), 4.67 (s, 2H), 4.12 (m, 2H), 3.91 (m, 4H), 3.72 (d, *J* = 10.9 Hz, 1H), 3.56 (d, *J* = 10. 5 Hz, 1H), 3.43 (m, 1H), 3.36 (s, 3H), 1.28 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.85 (s, 9H), 0.72 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 176.1, 136.6,

125.1, 109.9, 94.6, 76.6, 64.6, 64.5, 63.1, 59.7, 56.6, 55.1, 52.5, 50.4, 47.3, 42.1, 41.4, 39.9, 38.9, 37.9, 32.7, 32.2, 31.5, 29.3, 27.1, 25.8 (3 X C), 23.7, 23.7, 21.8, 21.6, 18.1, 14.2, 12.3, –5.66 (2 X C).

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-21-hydroxy-3-(methoxymethoxy)-27-norcholest-5-en-25-one, cyclic 1,2-ethanediyl acetal (11). To a cold (0 °C) diethyl ether (10 mL) solution of steroid **10** (520 mg, 0.78 mmol) was added a 2 M THF solution of $LiAlH_4$ (2 mL, 4 mmol) and the reaction was stirred at room temperature for 4 h. The reaction mixture was cooled and water (0.5 mL) was added in small drops and the mixture was stirred for 20 min. To that mixture, 5 M aqueous NaOH (2 mL) was added and the mixture was stirred for 20 min. To the mixture was added water (2 mL) and stirring was continued for 1 h. The supernatant ethereal solution was decanted, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil. The crude product was purified by column chromatography (silica gel eluted with 20-30% EtOAc in hexane) to yield steroid **11** (450 mg, 93%): ^{1}H NMR(400 MHz, $CHCl_3$) δ 5.55 (b s, 1H), 4.69 (s, 2H), 3.94 (m, 4H), 3.74 (d, *J* = 10.6 Hz, 1H), 3.69 (m, 2H), 3.58 (d, *J* = 10.6 Hz, 1H), 3.47 (m, 1H), 3.37 (s, 3H), 1.32 (s, 3H), 0.87 (s, 9H), 0.87 (s, 3H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.6, 125.2, 110.1, 94.6, 76.6, 64.6, 63.0, 62.5, 57.2, 55.2, 50.5, 50.2, 42.4, 42.2, 41.5, 39.9, 39.6, 39.5, 32.7, 32.6, 31.5, 29.5, 29.3, 27.7, 25.8, 24.1, 23.7, 21.8, 20.7, 18.1, 12.4, –5.61, –5.63.

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-21-[(methylsulfonyl)oxy]-27-norcholest-5-en-25-one, cyclic 1,2-ethanediyl acetal (12). To a CH_2Cl_2 solution (8 mL) of steroid **11** (400mg, 0.64 mmol) were added triethyl amine (1 mL) followed by mesyl chloride (0.16 mL, 2 mmol) and the reaction was stirred at room temperature for 2 h. The reaction was quenched with aqueous saturated $NaHCO_3$ (5 mL) and the biphasic solution was allowed to stir for 30 min. The product was extracted into CH_2Cl_2 and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 20-30% EtOAc in hexane) to give steroid **12** (445 mg, 99%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.53 (b s, 1H), 4.69 (s, 2H), 4.35 (m, 1H), 4.18 (m, 1H), 3.74 (d, *J* = 10.6 Hz, 1H), 3.56 (d, *J* = 10.6 Hz 1H), 3.46 (m, 1H), 3.35 (s, 3H), 2.99 (s, 3H), 1.29 (s, 3H), 0.86 (s, 9H), 0.72 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.4, 125.0, 109.8, 94.6, 76.5, 70.1, 64.5, 63.1, 57.0, 55.1, 50.3, 49.9, 42.2, 41.3, 39.8, 39.6, 39.4, 39.3, 37.2, 32.7, 32.6, 31.3, 29.3, 29.21, 27.4, 25.8 (3 X C), 23.9, 23.7, 21.7, 20.0, 18.1, 12.4, –5.7 (2 X C).

(3β)-19-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-(methoxymethoxy)-27-norcholest-5-en-25-one, cyclic 1,2-ethanediyl acetal (13). To a cold (0 °C) diethyl ether (10 mL) solution of steroid **12** (350 mg, 0.50 mmol) was added a 2 M THF solution of $LiAlH_4$ (2 mL, 4mmol) and the reaction was stirred at room temperature for 4 h. The reaction mixture was cooled and water (0.5 mL) was added in small drops and the mixture was stirred for 20 min. To that mixture, 5 M aqueous NaOH (2 mL) was added and the mixture was stirred for 20 min. To that solution was added water (2 mL) and stirring was continued for 1 h. The supernatant ethereal solution was decanted, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 10-15% EtOAc in hexane) to yield steroid **13** (290 mg, 96%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.54 (b s, 1H), 4.68 (s, 2H), 3.93 (m, 4H), 3.73 (d, *J* = 10.6 Hz, 1H), 3.58 (d, *J* = 10.6 Hz, 1H), 3.45 (m, 1H), 3.36 (s, 3H), 1.31 (s, 3H), 0.87 (s, 9H), 0.70 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.6, 125.2, 110.2, 94.6, 76.6, 64.6, 64.5, 63.0, 57.3, 56.0, 55.1, 50.5, 42.5, 41.4, 40.2, 39.9, 39.6, 36.1, 35.7, 32.7, 32.6, 31.6, 30.3, 29.3, 28.2, 25.8 (3 X C), 24.2, 23.7, 21.8, 20.6, 18.6, 18.1, 12.1, –5.63 (2 X C).

(3β)-19-Hydroxy-3-(methoxymethoxy)-27-norcholest-5-en-25-one, cyclic 1,2-ethanediyl acetal (14). Steroid **13** (121 mg, 0.2 mmol) and 1 M tetra *t*-butylammonium fluoride in THF (1 mL) and THF (8 mL) were heated at reflux for 1h. The reaction was cooled, diluted with water and the product extracted into CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give a crude oil, which was purified by flash column chromatography (silica gel) to give steroid **14** (90 mg, 92%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.73 (b s, 1H), 4.67 (s, 2H), 3.92 (m, 4H), 3.83 (d, *J* = 10.6 Hz, 1H), 3.58 (d, *J* = 10.9 Hz, 1H), 3.46 (m, 1H), 3.35 (s, 3H), 1.30 (s, 3H), 0.91 (d, *J* = 7 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 135.5, 127.3, 110.2, 94.7, 76.5, 64.5, 62.7, 57.6, 56.0, 55.1, 50.4, 42.5, 41.7, 40.0, 39.6, 39.6, 36.1, 35.7, 33.33, 33.29, 31.2, 29.4, 29.3, 28.2, 25.6, 24.0, 23.7, 21.7, 20.6, 18.6, 12.2.

(3β)-25,25-[1,2-Ethanediylbis(oxy)]-3-(methoxymethoxy)-27-norcholest-5-en-19-al (15). Steroid **14** (49 mg, 0.1 mmol), pyridinium chlorochromate (86 mg, 0.4 mmol) and CH_2Cl_2 (8 mL) were stirred at room temperature for 3 h. The mixture was diluted with hexane (10 mL) and the supernatant liquid was passed through a silica gel column (eluted with 25-30% EtOAc in hexane) to give steroid **15** (39 mg, 80%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 5.84 (b s, 1H), 4.65 (s, 2H), 3.92 (m, 4H), 3.41 (m, 1H), 3.34 (s, 3H), 1.30 (s, 3H), 0.89 (d, *J* = 6.7 Hz, 3H), 0.61 (s, 3H); ^{13}C NMR(100

MHz, $CDCl_3$) δ 205.3, 132.6, 127.5, 110.1, 94.7, 75.9, 64.6, 64.6, 56.4, 55.9, 55.1, 53.7, 48.9, 42.2, 41.0, 39.6, 39.6, 36.0, 35.6, 32.9, 31.2, 30.4, 29.9, 28.1, 23.9, 23.7, 22.2, 20.5, 18.6, 11.8.

(3β)-10-Ethynyl-3-(methoxymethoxy)-19,27-dinorcholest-5-en-25-one, cyclic 1,2-ethanediyl acetal (16). To a stirred suspension of chloromethyl triphenyl phosphonium chloride (347 mg, 1mmol) in THF (6 ml) was added 2.5 M *n*-butyl lithium in hexane (0.4 ml, 1 mmol) and the reaction was refluxed for 30 min. The reaction was cooled, a THF solution (3 mL) of steroid **15** (39 mg, 0.08 mmol) was added and the reaction was heated to reflux for another 2 h. The reaction mixture was cooled, quenched with saturated aqueous NH_4Cl and the product extracted into EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 10-25% EtOAc in hexane) to give the 10-chloroalkene (–CH=CHCl), which was immediately subjected to elimination without any characterization.

To a cold (-78 °C) solution of *n*-butyl lithium (6 mL, 2.5 mmol in THF), was added the crude 10-chloroalkene in THF (3 mL) and the reaction was slowly warmed to room temperature over a period of 40 min and stirred at room temperature for another 15 min. The reaction was cooled, carefully quenched with cold water and the product extracted into EtOAc. The combined EtOAc extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 10-20% EtOAc in hexane) to give steroid **16** (21 mg, 55 %) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.48 (b s, 1H), 4.70(s, 2H), 3.94 (m, 4H), 3.43 (m, 1H), 3.38 (s, 3H), 1.32 (s, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.8, 123.3, 110.2, 94.7, 87.0, 76.0, 72.0, 64.6, 56.0, 55.8, 55.2, 48.2, 42.4, 40.3, 39.7, 39.7, 39.3, 36.1, 35.7, 35.7, 33.2, 31.7, 29.5, 28.2, 24.2, 23.7, 22.7, 20.6, 18.6, 11.7.

(3β)-10-Ethynyl-3-(methoxymethoxy)-19,27-dinorcholest-5-en-25-one (17). A mixture of steroid 16l (20 mg), acetone (5 mL) and PTSA (20 mg) was stirred at room temperature for 2 h. The reaction was made basic with aqueous $NaHCO_3$ and the acetone was removed. The residue was diluted with water and the product was extracted into CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with 10-20% EtOAc in hexane) to give steroid **17** (18 mg, 99%) which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.48 (b s, 1H), 4.70(s, 2H), 3.43 (m, 1H), 3.38 (s, 3H), 2.26 (s, 1H), 2.14 (s, 3H),

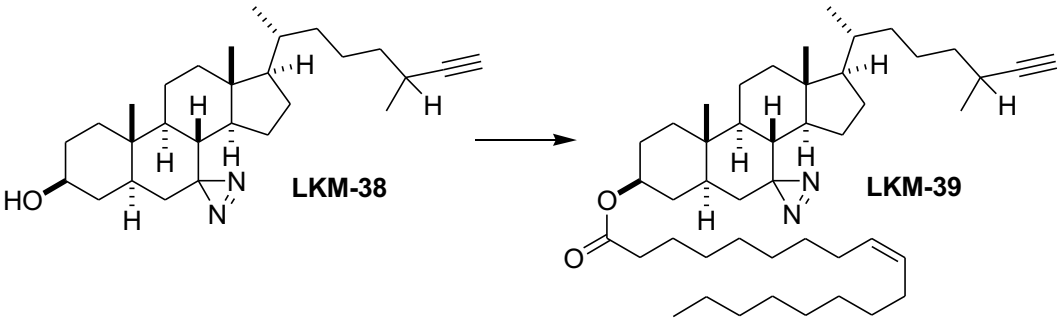
0.94 (d, J = 6.3 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 209.4, 136.8, 123.2, 94.7, 87.0, 76.0, 72.0, 55.8, 55.7, 55.2, 48.2, 44.3, 42.4, 40.3, 39.6, 39.3, 35.7, 35.6, 35.4, 33.2, 31.6, 29.9, 29.7, 29.5, 28.2, 24.2, 22.6, 20.3, 18.6, 11.7.

(3β)-25-Azi-10-ethynyl-3-(methoxymethoxy)-19,27-dinorcholest-5-ene (18). Steroid **17** (18 mg, 0.04 mmol) and 7 M ammonia in MeOH(4 mL) was stirred at room temperature under a nitrogen atmosphere for 4 h. Hydroxylamine-O-sulfonic acid (4.5 mg, 0.04 mmol) dissolved in MeOH (1 mL) was added and the resulting mixture was stirred at room temperature for 16 h. The solid floating in the solution was removed by filtration and the filter-cake was washed with MeOH. The solvent from the combined filtrate and the washings was removed under reduced pressure to give the intermediate 20-diaziridine as a residue which was not characterized and was immediately converted to steroid **18** as described below.

The crude 20-diaziridine was dissolved in CH_2Cl_2 (5 mL) and triethyl amine (0.2 mL) was added to the solution. To the stirred solution was added in small portions I_2 (50 mg) dissolved in CH_2Cl_2 (2 mL) until a brown color persisted. After few min, a few drops of 5% aqueous sodium thiosulfate was added to decolorize the solution and the resulting mixture was diluted with water (10 mL) and the product extracted into CH_2Cl_2 (3 x 10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 5-15% EtOAc in hexane) to give steroid **18** (8 mg, 44%) as an off-white solid which had: ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.48 (b s, 1H), 4.70(s, 2H), 3.42 (m, 1H), 3.38 (s, 3H), 2.26 (s, 1H), 1.00 (s, 3H), 0.91 (d, J = 6.2 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.8, 123.3, 110.0, 94.7, 87.0, 76.0, 72.0, 55.8, 55.8, 55.2, 48.2, 40.3, 39.6, 39.3, 35.7, 35.6, 35.5, 34.7, 33.2, 31.7, 29.5, 28.2, 25.9, 24.2, 22.6, 20.6, 20.0, 18.6, 11.7.

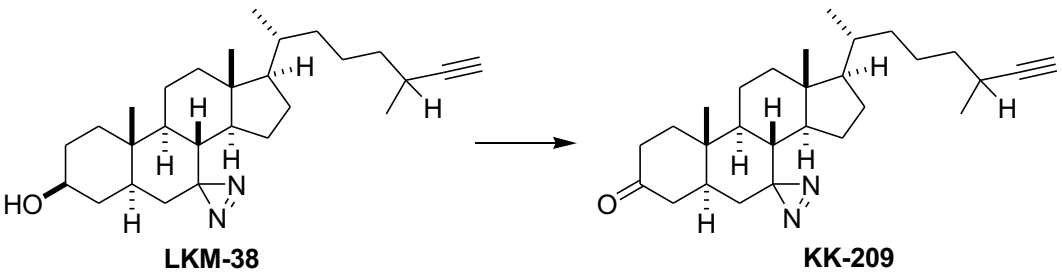
(3β)-25-Azi-10-ethynyl-19,27-dinorcholest-5-en-3-ol (19, KK-175). Steroid **18** (8 mg, 0.017 mmol) and 10% dry HCl in MeOH (5 mL) was stirred at room temperature for 4 h. The reaction mixture was carefully neutralized by adding aqueous $NaHCO_3$ and the product was extracted into CH_2Cl_2 (3 x 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with 20- 30% EtOAc in hexane) to give steroid **19** (**KK-175**, 6 mg, 83%) which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (b s, 1H), 3.51 (m, 1H), 2.26 (s, 1H), 0.99 (s, 3H), 0.91 (d, J = 6.2 Hz, 3H), 0.71 (s, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 136.7, 123.3, 87.1, 72.0, 70.9, 55.8, 55.8, 48.1, 42.4, 40.0, 39.3, 35.7, 35.6, 35.5, 34.7, 33.2, 32.3, 31.6, 28.2, 26.0, 24.2, 22.7, 20.5, 20.0, 18.6, 11.9; IR(film, cm^{-1}) 3427, 2937, 2865, 1585, 1464.

Synthesis of LKM-39



(3β,5α)-20-(4-Methyl-5-hexyn-1-yl)-spiro[pregnane-7,3'-[3*H*]diazirin]-3-ol, oleic acid ester (LKM-39). LKM-38 (20 mg, 0.047 mmol), prepared as previously described (*Science,* 2017, **355**, 1306; supplemental material), oleic acid (22 µL, 0.071 mmol) and DMAP (9 mg, 0.071 mmol) were added to CH_2Cl_2 (5 mL) at 0 °C. *N*,*N*′-dicyclohexylcarbodiimide (11.3 mg, 0.055 mmol) was dissolved in CH_2Cl_2 (0.1 mL) and added dropwise. The reaction was brought to room temperature and stirred for 16 h. Upon completion the reaction mixture was diluted with CH_2Cl_2 (10 mL), filtered, washed with 1 N HCl (5 mL), water (5 mL) and sat. aqueous $NaHCO_3$ (5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (silica gel eluted with EtOAc in hexane, gradient elution), to yield the product **LKM-39** as a colorless oil (29 mg 0.042 mmol, 90%) which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (m, 2H), 4.72 (m, 1H), 2.43-2.35 (m, 1H), 2.25 (t, 2H, *J* = 7.6 Hz), 2.03 (d, 1H, *J* = 2.4 Hz, CCH), 1.16 (d, 3H, *J* = 6.8 Hz), 0.94 (s, 3H), 0.89-0.87 (m, 6H), 0.79-0.66 (m, 1H), 0.59 (s, 3H), 0.34-0.24 (m, 1H), -0.01 (dd, 1H, *J* = 14.0 Hz, *J* = 3.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 130.2, 129.9, 89.5, 72.9, 68.2, 54.6, 52.4, 50.0, 43.4, 42.5, 38.9, 37.4, 37.3, 36.5, 36.0, 36.0, 35.8, 35.6, 34.9, 33.4, 32.2, 32.1, 30.0, 29.9, 29.7, 29.5, 29.4, 29.3, 29.3, 28.1, 27.5, 27.4, 27.4, 25.9, 25.2, 25.0, 23.8, 22.9, 21.1, 21.1, 19.0, 14.3, 12.1, 11.6.

Synthesis of KK-209



(3β,5α)-20-(4-Methyl-5-hexyn-1-yl)-spiro[pregnane-7,3'-[3*H*]diazirin]-3-one (KK-209). LKM-38, (12 mg, 0.028 mmol), prepared as previously described (*Science,* 2017, **355**, 1306; supplemental material) and PCC (21 mg, 0.1 mol) in dichloromethane (2 mL) were stirred at room temperature for 2 hr. Hexane (3 mL) was added and after stirring for few min the supernatant liquid was transferred to a silica gel column and eluted with 10-20% EtOAc in hexane to give the product (**KK-209**, 9 mg, 75%) as a white solid which had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.5-0.8 (m), 1.16 (d, 3H, J = 7.0 Hz), 1.12 (s, 3H), 0.89 (d, 3H, J = 6.7 Hz), 0.62 (s, 3H), 0.29 (m, 1H), 0.06 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 210.61, 89.31, 68.02, 54.29, 51.68, 49.66, 43.95, 43.68, 43.11, 38.63, 37.88, 37.79, 37.09, 36.97, 35.94, 35.89, 35.54, 35.36, 31.75, 27.87, 25.66, 24.71, 23.60, 21.19, 20.90, 18.73, 11.94, 10.66.