SUPPORTING INFORMATION

Efficient Syntheses of Cocaine Vaccines and their *In Vivo* Evaluation

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I. Experimental Procedures

6-((1R,2R,3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxamido)hexanoic acid (GNE: 5). To a solution of **7** (55.1 mg, 0.112 mmol) in EtOAc (1 mL) was added Pd/C (6 mg) at rt. After stirring for 16 h under H_2, additional Pd/C (4 mg) was added. After stirring for an additional 3 h, the reaction mixture was filtered through a Celite® pad and evaporated *in vacuo* to afford crude GNE **5**, which was used in the next step without further purification. The spectroscopic data for **5** was collected after purification by preparative TLC (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 5:1:0.1). ^{1}H-NMR (600 MHz, $CDCl_3$) δ9.29 (br s, 1H), 7.96 (d, *J*=7.8 Hz, 2H), 7.53 (t, *J*=7.2 Hz, 1H), 7.41 (t, *J*=7.2 Hz, 2H), 5.34 (dt, *J*=11.4, 7.2 Hz, 1H), 3.65 (br s, 1H), 3,45 (br s, 1H), 3.42-3.37 (m, 1H), 3.26-3.21 (m, 1H), 3.08 (br s, 1H), 2.43 (s, 3H), 2.33-2.29 (m, 2H), 2.20-2.15 (m, 2H), 2.12-2.08 (m, 2H), 1.90-1.79 (m, 2H), 1.82-1.79 (m, 1H), 1.68-1.62 (m, 2H), 1.61-1.56 (m, 2H), 1.44-1.38 (m, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 176.1, 171.4, 165.8, 133.1, 129.9, 129.7 (2C), 128.4 (2C), 65.5, 63.9, 61.0, 40.0, 38.7, 35.3, 33.6, 29.7, 29.0, 26.1, 25.4, 24.7, 24.2; HRMS (ESI) calc'd for $C_{22}H_{31}N_2O_5$ (M+H$^+$) 403.2233, found 403.2222.

6-((1R,2S,3S,5S)-3-benzamido-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxamido)hexanoic acid (GND: 6). To a solution of **14** in MeOH (300 µL) was added Pd/C (1 mg) at rt. After stirring for 18 h under H_2, the reaction mixture was filtered through a Celite® pad and evaporated *in vacuo* to afford crude GND **6**, which was used in the next step without further purification. The spectroscopic data for **6** was collected after purification by preparative TLC (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 5:1:0.1). ^{1}H-NMR (500 MHz, CD_3OD) δ 7.81 (d, *J*=8.0 Hz, 2H), 7.53 (t, *J*=7.5 Hz, 1H), 7.45 (t, *J*=8.0 Hz, 2H), 4.55 (dt, *J*=12.5, 6.0 Hz, 1H), 3.91 (br d, *J*=5.5 Hz, 1H), 3.81 (br s, 1H), 3.27-3.21 (m, 1H), 3.17-3.12 (m, 1H), 3.04 (br d, *J*=4.5 Hz, 1H), 2.68 (s, 3H), 2.50-2.32 (m, 4H), 2.09-2.06 (m, 4H), 2.00 (ddd, *J*=14.5, 6.0, 4.0 Hz, 1H), 1.68-1.55 (m. 1H), 1.53-1.47 (m, 2H), 1.46-1.39 (m, 2H), 1.29-1.22 (m, 3H); ^{13}C-NMR (75 MHz, D_2O) δ 182.9, 171.9, 170.3, 132.1, 131.9, 128.1 (2C), 126.5 (2C), 64.0, 62.4, 45.4, 40.7, 38.5, 37.7, 36.4, 31.7, 27.4, 25.2, 24.5, 22.9, 22.0; HRMS (FAB) calc'd for $C_{22}H_{32}N_3O_4$ (M+H$^+$) 402.2393, found 402.2396.

(1R,2R,3S,5S)-2-((6-(benzyloxy)-6-oxohexyl)carbamoyl)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl benzoate (7). A solution of cocaine (**1**) (65.8 mg, 0.217 mmol) in 1,4-dioxane/H_2O (4 mL, 1:1, v/v) was microwave irradiated (130 °C, 90 psi, 40 W) with stirring. After 2 h, the reaction mixture was filtered, and the filtrate was evaporated *in vacuo* to afford crude benzoylecgonine (**3**). The crude solid was dissolved into THF (2 mL), and then benzyl 6-aminohexanoate (72.0 mg, 0.325 mmol), DMTMM (72.0 mg, 0.260 mmol) and triethylamine (30.0 µL, 0.215 mmol) were added at rt. After stirring for 3 h, additional benzyl 6-aminohexanoate (80.0 mg, 0.362 mmol) and DMTMM (80.0 mg, 0.289 mmol) were added to the mixture. After stirring for an additional 10 h, the reaction mixture was quenched with 0.5 M NaOH. The aq. layer was extracted with 10% MeOH in CH_2Cl_2, and the combined organic extracts were washed with sat. NH_4Cl, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude oil was purified by preparative TLC (SiO_2; CH_2Cl_2:MeOH = 9:1) to afford 55.1 mg of GNE benzyl ester **7** (52% over 2 steps). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.49 (br s, 1H), 7.96 (d, *J*=7.2 Hz, 2H), 7.48 (t, *J*=7.2 Hz, 1H), 7.38 (t, *J*=7.8 Hz, 2H), 7.36-7.32 (m, 5H), 5.31 (dt, *J*=11.4, 7.2 Hz, 1H), 5.10 (s, 2H), 3.38-3.31 (m, 1H), 3,31 (br s, 1H), 3.27-3.19 (m, 2H), 2.93 (br s, 1H), 2.33 (t, *J*=7.2 Hz, 2H), 2.29 (s, 3H), 2.22-2.16 (m, 1H), 2.12-2.09 (m, 2H), 1.98 (br t, *J*=10.2 Hz, 1H), 1.81 (br t, *J*=9.6 Hz, 1H), 1.76 (q, *J*=5.4 Hz, 1H), 1.70-1.64 (m, 2H), 1.61-1.55 (m, 2H), 1.42-1.37 (m, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 173.4, 171.1, 165.9, 136.0, 132.9, 130.1, 129.7 (2C), 128.5 (2C), 128.3 (2C), 128.2, 128.1 (2C), 66.6, 65.8, 63.5, 60.6, 40.3, 38.9, 36.6, 34.1, 30.6, 29.4, 26.6, 24.8, 24.6, 23.1; HRMS (ESI) calc'd for $C_{29}H_{37}N_2O_5$ (M+H$^+$) 493.2702, found 493.2697.

GNE-TT Immunoconjugate (8). To a solution of **5** (2.6 mg, 6.4 µmol) in DMF/H_2O (130 µL, 9:1, v/v) was added sulfoNHS (4.2 mg, 0.019 mmol) and EDCI (3.7 mg, 0.019 mmol) at rt. After stirring for 15 h, an aliquot (55 µL) of the reaction mixture was added to a solution of TT (UMass Biologics) in pH 7.4 PBS (1 mL, 1.39 mg/mL). Another aliquot (20 µL) of the reaction mixture was added to BSA (Imject™) in pH 7.4 PBS (450 µL, 1 mg/mL). After stirring each reaction mixture at 4 °C for 20 h, the mixtures were dialyzed against pH 7.4 PBS at rt using a Slide-A-Lyzer 10 K MWCO dialysis device to afford GNE-TT **8** and GNE-BSA. The buffer was exchanged every 2 h for 6 h, and then dialysis was continued for 12 h at 4 °C.

Methyl (1R,5S)-3-amino-8-methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylate (10). A solution of methylecgonone bitartrate (**9**, 0.301 mg, 0.864 mmol) in sat. NH_3 in MeOH (6 mL) was microwave irradiated (100 °C, 150 psi, 300 W) with stirring. After 4.5 h, the solvent was evaporated *in vacuo*. The crude oil was purified by flash column chromatography (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 9:1:0.1) to afford 0.101 g of enamine **10** (59%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.82 (d, *J*=5.1 Hz, 1H), 3.67 (s, 3H), 3.36 (t, *J*=5.7 Hz, 1H), 2.73 (dd, *J*=17.1, 4.9 Hz, 1H), 2.31 (s, 3H), 2.19-2.01 (m, 3H), 1.81-1.68 (m, 3H), 1.53-1.42 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.2, 153.8, 150.5, 57.9, 57.4, 50.4, 36.6, 34.9, 33.9, 29.0; HRMS (FAB) calc'd for $C_{10}H_{17}N_2O_2$ (M+H$^+$) 197.1290, found 197.1287.

Methyl (1R,3S,5S)-3-benzamido-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (12). To a solution of **10** (0.212 g, 1.08 mmol) in MeOH (1.9 mL) was added bromocresol green (trace) and $NaBH_3CN$ (67.0 mg, 1.07 mmol) at rt. Then,

2 M HCl in 1,4-dioxane/MeOH was added at rt to adjust the pH of the reaction mixture to 4. After stirring for 12 h, additional $NaBH_3CN$ (80.0 mg, 1.27 mmol) and 2 M HCl in 1,4-dioxane/MeOH was added. After 11 h, more $NaBH_3CN$ (70.0 mg, 1.11 mmol) and 2 M HCl in 1,4-dioxane/MeOH was added. After 10 h, the solvent was evaporated *in vacuo*. The crude oil was dissolved into a mixture of H_2O/1,4-dioxane (3 mL, 1:1, v/v), and then benzoyl chloride (0.230 mL, 1.96 mmol) and $NaHCO_3$ (0.420 mg, 4.99 mmol) were added at rt. After stirring for 18 h, the reaction mixture was quenched with H_2O and extracted 3x with 20% MeOH in CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude oil was purified by flash column chromatography (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 20:1:0.02 to 5:1:0.02) and then re-purified by preparative TLC (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 15:1:0.1) to afford 65.3 mg of benzamide **12** (20% over 2 steps). ^{1}H-NMR (300 MHz, CD_3OD) δ 7.84-7.78 (m, 2H), 7.60-7.48 (m, 3H), 4.58 (dt, *J*=11.4, 6.2 Hz, 1H), 3.67 (s, 3H), 3.50-3.45 (m, 1H), 3.32-3.28 (m, 1H), 3.06 (dd, *J*=11.5, 2.7 Hz, 1H), 2.45 (s, 3H), 2.31-1.58 (m, 8H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.5, 166.8, 134.5, 131.3, 128.4, 126.9, 62.8, 60.4, 51.9, 49.6, 43.2, 38.5, 35.6, 26.2, 23.6; HRMS (FAB) calc'd for $C_{17}H_{22}N_2O_3$ (M+H$^+$) 303.1709, found 303.1724.

Benzyl 6-((1R,2S,3S,5S)-3-benzamido-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxamido)hexanoate (14). A solution of **12** (20.9 mg, 0.691 mmol) in H_2O (890 μL) was refluxed for 12 h, and then the solvent was evaporated in *vacuo*. The crude oil was dissolved in CH_2Cl_2 (420 μL), and benzyl 6-aminohexanoate toluenesulfonic acid salt (32.0 mg, 0.813 mmol), EDCI (25.0 mg, 0.130 mmol), triethylamine (13.0 μL, 93.1 μmol) and DMAP (1.00 mg, 8.10 μmol) were added at rt. After stirring for 22 h, the reaction mixture was quenched with H_2O and extracted 3x with 10% MeOH in CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residual oil was purified by preparative TLC (SiO_2; CH_2Cl_2:MeOH:aq. NH_3 = 7:1:0.1) to afford 5.50 mg of benzyl ester **14** (16% over 2 steps). ^{1}H-NMR (600 MHz, CD_3OD) δ 7.74 (d, *J*=7.2 Hz, 2H), 7.49 (tt, *J*=7.2, 1.8 Hz, 1H), 7.42 (t, *J*=7.2 Hz, 2H), 7.35-7.29 (m, 5H), 5.11-5.07 (m, 2H), 4.41 (dt, *J*=12.6, 6.0 Hz, 1H), 3.41 (br s, 1H), 3.30-3.27 (m, 2H), 3.22-3.16 (m, 1H), 2.72 (br s, 1H), 2.36 (t, *J*=7.2 Hz, 2H), 2.28 (br s, 3H), 2.22-2.21 (m, 1H), 2.18-2.13 (m, 1H), 2.02 (br s, 1H), 1.83 (br s. 1H), 1.83-1.77 (m, 2H), 1.68-1.61 (m, 3H), 1.55-1.53 (m, 2H), 1.40-1.37 (m, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 175.0, 174.5, 169.9, 137.7, 135.8, 132.6, 130.0 (2C), 129.5 (2C), 129.2 (3C), 128.3 (2C), 67.1, 65.2 (2C), 43.0 (2C), 39.8 (2C), 34.8, 30.1 (2C), 27.5 (2C), 25.6 (2C); HRMS (FAB) calc'd for $C_{29}H_{38}N_3O_4$ (M+H$^+$) 492.2862, found 492.2873.

GND-TT Immunoconjugate (15). To a solution of **6** (2.6 mg, 6.4 μmol) in DMF/H_2O (130 μL, 9:1, v/v) was added sulfoNHS (4.2 mg, 0.019 mmol) and EDCI (3.7 mg, 0.019 mmol) at rt. After stirring for 15 h, an aliquot (55 μL) of the reaction mixture was added to TT (UMass Biologics) in pH 7.4 PBS (1 mL, 1.39 mg/mL). Another aliquot (20 μL) of the reaction mixture was added to BSA (Imject^{TM}) in pH 7.4 PBS (439 μL, 1 mg/mL). After stirring each reaction mixture at 4 °C for 20 h, the mixtures were dialyzed against pH 7.4 PBS at rt using a Slide-A-Lyzer 10 K MWCO dialysis device to afford GND-TT **15** and GND-BSA. The buffer was exchanged every 2 h for 6 h, and then dialysis was continued for 12 h at 4 °C.

Mass Spectrometry Analysis. In order to quantify the hapten copy number (hapten density) for GNE-TT and GND-TT, samples were submitted for MALDI-ToF MS (Applied Biosystems Voyager DE) analysis and compared to unconjugated protein. Copy numbers were calculated using the following formula: Copy # = (Conjugated MW – Unconjugated MW) / Hapten MW.

Animals and Vaccinations. 6-8 week old male Swiss Webster mice (n = 6/group) were obtained from Taconic Farms (Germantown, NY). Mice were group-housed in an AAALAC-accredited vivarium containing temperature- and humidity-controlled rooms, with mice kept on a reverse light cycle (lights on: 9PM-9AM). All experiments were performed during the dark phase, generally between 1PM-4PM. General health was monitored by both the scientists and veterinary staff of The Scripps Research Institute, and all studies were performed in compliance with the Scripps Institutional Animal Care and Use Committee (Protocol #08-0127) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. GNE-TT and GND-TT immunoconjugates in PBS pH 7.4 (48 μL, 1.2 mg/mL) were formulated with alum (Alhydrogel®, Invivogen, 100 μL, 10 mg/mL) and CpG ODN 1826 (Eurofins MWG Operon, 5 μL, 10 mg/mL). All vaccine injections were conducted intraperitoneally (150-200 μL) on days 0, 14, 28, 66 and 88. No adverse reactions were observed and all mice maintained a healthy weight throughout the vaccine trial. Blood sampling was performed via tail-tip amputation (<1 cm) in order to collect 100-150 μL whole blood on days 20, 35, 73 and 95. Whole blood samples were centrifuged at 10000 rpm for 10 min to isolate serum.

Enzyme-Linked Immunosorbent Assay (ELISA). PBS pH 7.4 was prepared from 10x powder (Fisher Science) and used throughout the assay, except for the washing steps which used ddH_2O. First, half-area, high-binding 96-well microtiter plates (Costar 3690) were coated with 25 ng of GNE-BSA or GND-BSA per well at 37 °C overnight, allowing the liquid to evaporate. Following blocking with 5% skim milk in PBS pH 7.4 at rt for 1 h, mouse serum was serially diluted 1:2 in 2% BSA in PBS pH 7.4 across the 12 columns starting at 1:300. After a 2 h incubation at rt, the plates were washed 10x with ddH_2O, then donkey anti-mouse IgG horseradish peroxidase (HRP) secondary (Jackson ImmunoResearch) was added at a 1:10000 dilution in 2% BSA in PBS pH 7.4 and incubated at rt for 2 h. After the incubation, 10x washing with ddH_2O was performed and 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (ThermoPierce)

was added. TMB was incubated for 10 min at rt, then the mixture was quenched with 2 M aq. H_2SO_4. Plates were allowed to incubate at rt for 15 min before their absorbance was read at 450 nm.

Competitive Radioimmunoassay (Competitive RIA). Dissociation constants (K_d) and antibody concentrations were determined using competitive RIA. Mouse sera were pooled and diluted into 2% BSA in PBS pH 7.4, giving a concentration that was determined to bind ca 30-50% ^{3}H-cocaine tracer (35.5 Ci/mmol (PerkinElmer, NET510100UC)). Diluted serum (60 µL) and cocaine tracer (60 µL) were added to the sample chamber of a 5 kDa MWCO 6-well Equilibrium Dialyzer (Harvard Apparatus), and cocaine hydrochloride salt at varying concentrations in 1% BSA in PBS pH 7.4 (120 µL) was added to the buffer chamber. After equilibration on a plate rotator (Harvard Apparatus) at rt for 22-26 h, a sample from each chamber (60 µL) was diluted into Ecolite(+)™ liquid scintillation cocktail (5 mL, MP Biomedicals), and the radioactivity was measured with a Beckman LS 6500 Scintillation Counter. K_ds and antibody concentrations were calculated according to Müller's procedure.[1]

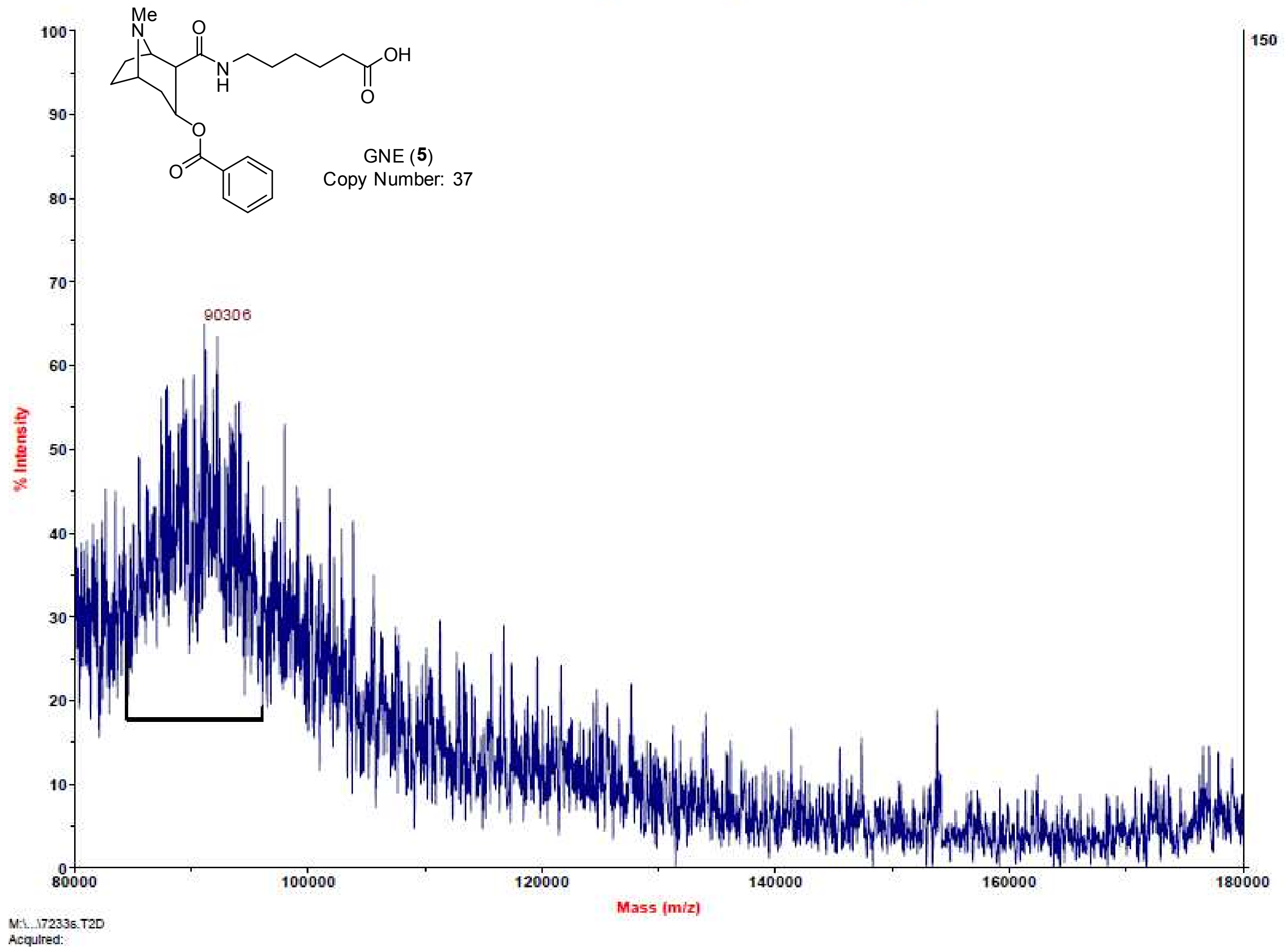
Hyperlocomotion Test. Mice were allowed to acclimate for one hour in a plastic cage (10.5 × 19 × 8 inch) with a clear ventilated acrylic top. Mice were then quickly removed and injected with either saline, 5, 10, or 20 mg/kg cocaine; cages were wiped down with dry paper towels to remove excess debris while mice were being injected. The mice were then returned to the cage to be recorded and tracked by an overhead camera using ANY-Maze video tracking software (Stoelting Co). Sessions were run during the middle of the dark cycle in a 4.6 × 4.6 m room with a single 60 W upward-directed light source (35-45 lux), and repeated after a two-day washout period until all mice received all cocaine doses. Distance travelled (m) and time spent immobile (s; 80% pixel consistency for at least 5 s threshold) were measured.

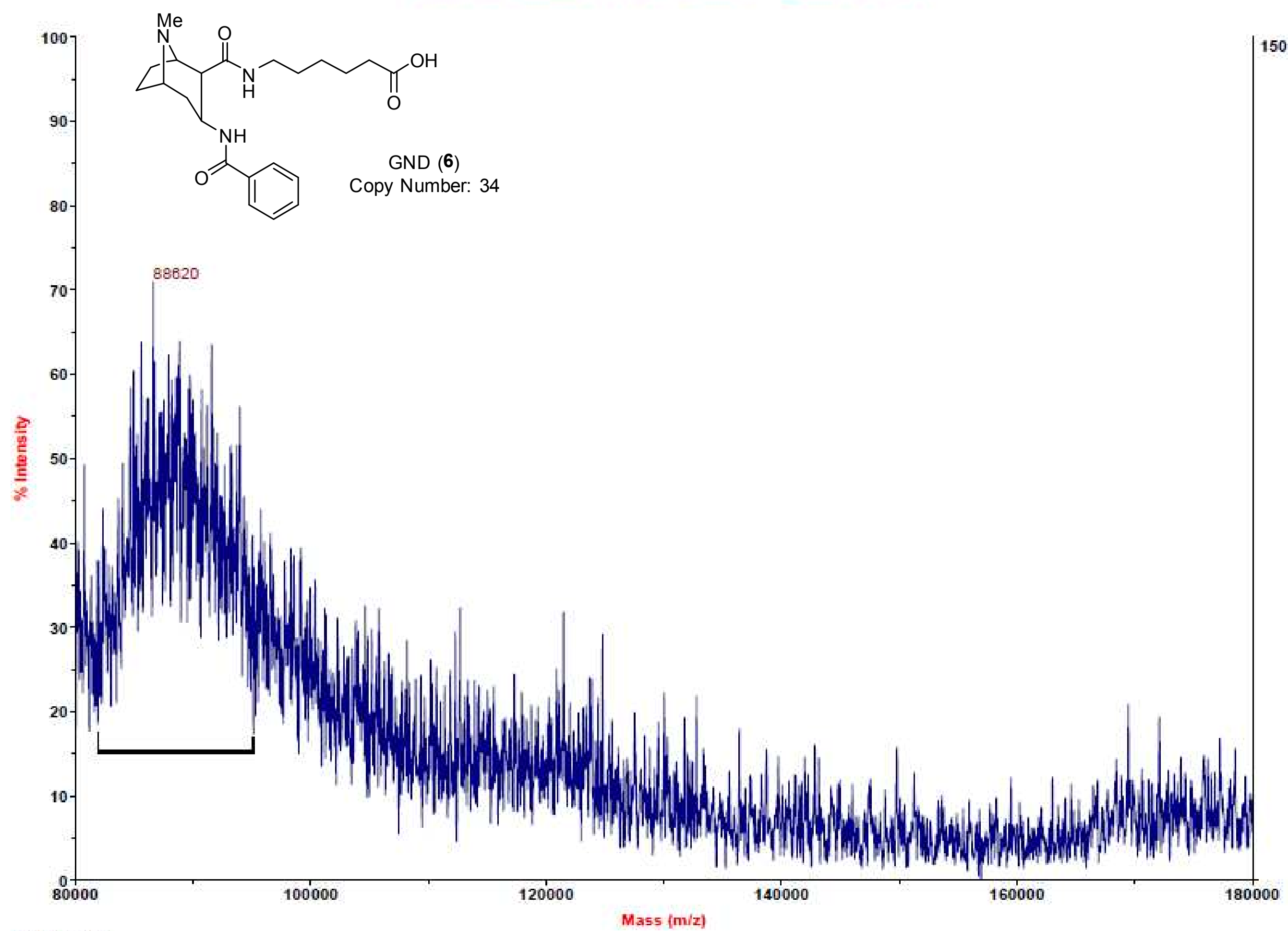
Pharmacokinetic Analysis. On day 81, three groups (GNE-TT, GND-TT, and naïve) of male Swiss Webster mice (n = 6) were injected intraperitoneally (5 mL/kg) with a solution containing 4.0 mg/mL of cocaine to provide a dose of 20 mg/kg. The animals were then returned to their home cage. Each animal had 2-3 blood samples taken via retro-orbital bleed at independent, preset periods of time (5, 15, 30, 60, 120, 240, or 1440 minutes) to generate n = 2 data points for each measured time point. The exact time points taken for each mouse were balanced with respect to group. Collected blood samples were stored on ice for 0.5-4 hours, then centrifuged at 10,000 rpm for 10 minutes to collect serum. The serum was stored at – 80 °C until analysis by LCMS. On the day of LCMS analysis, 10 µL of aq. NaF (600 µM) and 30 µL of d^3-cocaine in MeCN (163 nM) was added to 20 µL of each sample aliquot. The mixtures were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was removed for LCMS analysis. A 5 µL aliquot of each sample was injected into an LCMS system equipped with an Agilent Poroshell 120 SB-C8 column using H_2O + 0.1% formic acid and MeCN + 0.1% formic acid as the mobile phases. The percentage of MeCN + 0.1% formic acid was linearly increased from 5 - 95% over a 10 minute run (150 µL/min), followed by a 10 minute wash phase at 5% MeCN + 0.1% formic acid. Deuterated (internal standard; IS) and non-deuterated masses were extracted in MassHunter and the resulting peaks were integrated. Non-deuterated peak sizes were normalized alongside the IS peak to account for variability across sample runs. Quantification of the serum concentrations for each drug was achieved through the use of a 5-point standard curve for cocaine, using blank mouse serum that had been spiked with known concentrations (0 nM – 600 nM) (Figure S1). The elimination rate constant (k_e) was calculated by plotting the natural log of the concentration over time during the terminal elimination phase, and determining the slope. The half-life was then calculated using the formula $Ln(2)/k_e$ (Table S1).[2]

Biodistribution Study. On day 101, three groups (GNE-TT, GND-TT, and Naïve) of male Swiss Webster mice (n = 6) were injected intraperitoneally (10 mL/kg) with a solution containing 2 mg/mL of cocaine to provide a dose of 20 mg/kg, then returned to their home cage. Fifteen minutes following the injection, the animals were fully anesthetized using nose cones constructed from a 50 mL Falcon® conical centrifuge tube (Corning, NY) containing gauze pads soaked in isoflurane. The animals were then rapidly decapitated using a guillotine, the brains were extracted, and trunk blood was collected. The blood was placed on ice for 0.5-2 h and then centrifuged at 10,000 RPM for 10 min to collect the serum. To each 300 µL aliquot of a serum, 120 µL of aq. NaF (600 µM) was added. The brain tissue was immediately flash frozen in a dry ice cooled acetone bath. Serum and brain samples were stored at –80 °C until extraction and LCMS analysis. For brain tissue extraction, 1 mL of aq. NaF (600 µM) was added to each whole frozen brain. The mixture was homogenized using a Bullet Blender® (Next Advance, NY) and then centrifuged at 10000 rpm for 20 min. To a 150 µL aliquot of supernatant was added 150 µL (326 nM) of d_3-cocaine in MeCN, and the mixture was Vortex® mixed for 1 min to equilibrate. The mixture was then extracted with an Oasis® PRiME HLB Extraction Cartridge, and the extracted solution was evaporated using a GENEVAC®. These tissue samples were reconstituted with 50 µL of MeCN and analyzed using the injection and run protocol described above, and quantified using a 5-point standard curve for cocaine (Figure S1).

Computational Analyses. Computational and statistical analysis was performed in GraphPad Prism 6 (La Jolla, CA). All values are reported as means ± SEM. For the ELISA assays, absorbance values were normalized to highest absorbance value per sample, and a curve was fit using the log (inhibitor) vs. normalized response – variable slope equation to determine the midpoint titer and standard errors.

II. MALDI Spectra for GND and GNE Vaccines




TOF/TOF™ Linear Spec #1=>SM5[BP = 3253.8, 4139]
Me
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OH
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GND (6)
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III. Standard Curve for Cocaine used in Pharmacokinetic & Biodistribution Studies

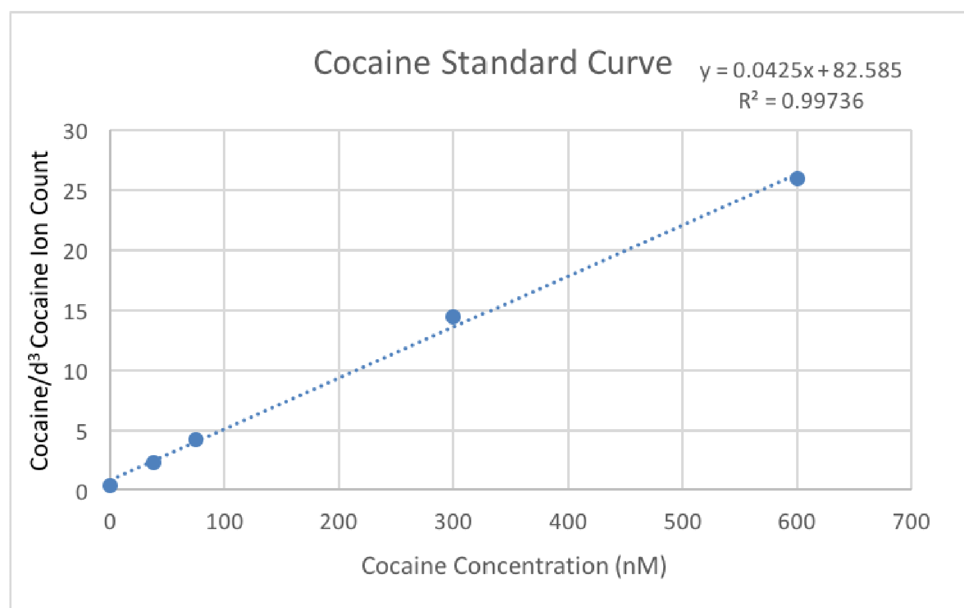

Figure S1. Standard curve generated using blank serum samples spiked with known concentrations of cocaine (0 – 600 nM). The linear regression obtained from this experiment was used to quantify the concentrations of cocaine in the LCMS pharmacokinetic and biodistribution studies.

IV. Calculated Pharmacokinetic Parameters

	Cocaine		
	$t_{1/2}$ (m)	C_{max} (ng/mL)	AUC_{0-24} (ng/mL*h)
GNE-TT	35.6	1382.3	516.5
GND-TT	34.5	1043.5	305.0
Naïve	3.3	206.2	13.1
	Fold-increase over Naïve		
	$t_{1/2}$ (m)	C_{max} (ng/mL)	AUC_{0-24} (ng/mL*h)
GNE-TT	10.7	6.7	39.4
GND-TT	10.4	5.1	23.3

Table S1. Calculated pharmacokinetic parameters for vaccinated and unvaccinated mice following intraperitoneal injection of 4 mg/kg cocaine.

V. Immobility Data from Cocaine-induced Hyperlocomotor Activity Assays

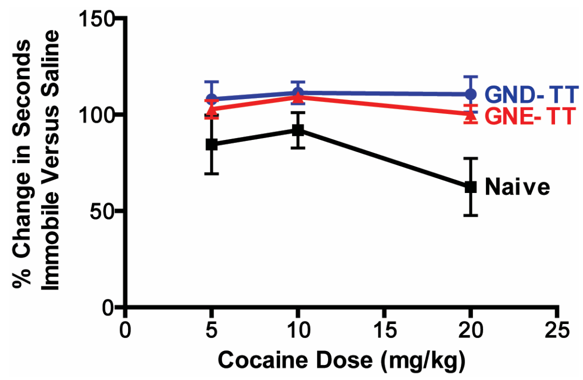

Figure S2. Percent change in time spent immobile over baseline during cocaine-induced hyperlocomotor assay in vaccinated mice (n = 6) at 0, 5, 10 and 20 mg/kg doses on days 42, 44, 46, and 48, respectively.

VI. RIA Binding Curves

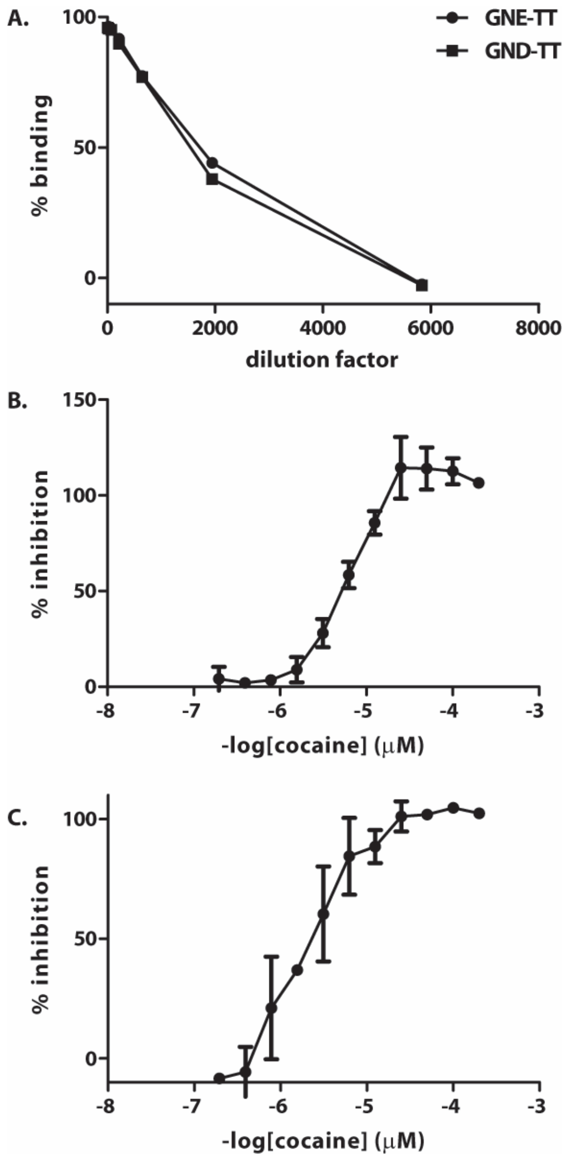

Figure S3. (A) Binding curves of pooled serum samples for cocaine-^{3}H for each treatment group. Optimal binding for competitive assay is 50%. (B) Competitive binding curve for GNE-TT. (C) Competitive binding curve for GND-TT. (B&C) IC_{50} values were calculated using Prism 6. Error bars represent SEM, n = 2.

VII. References

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