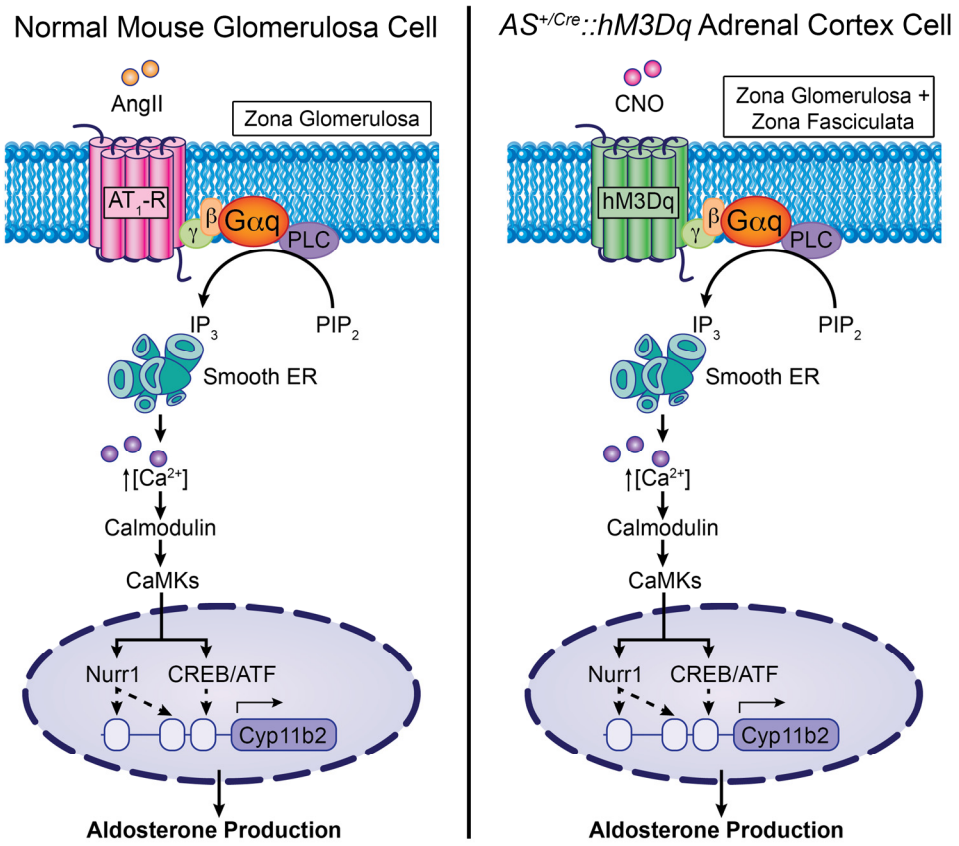
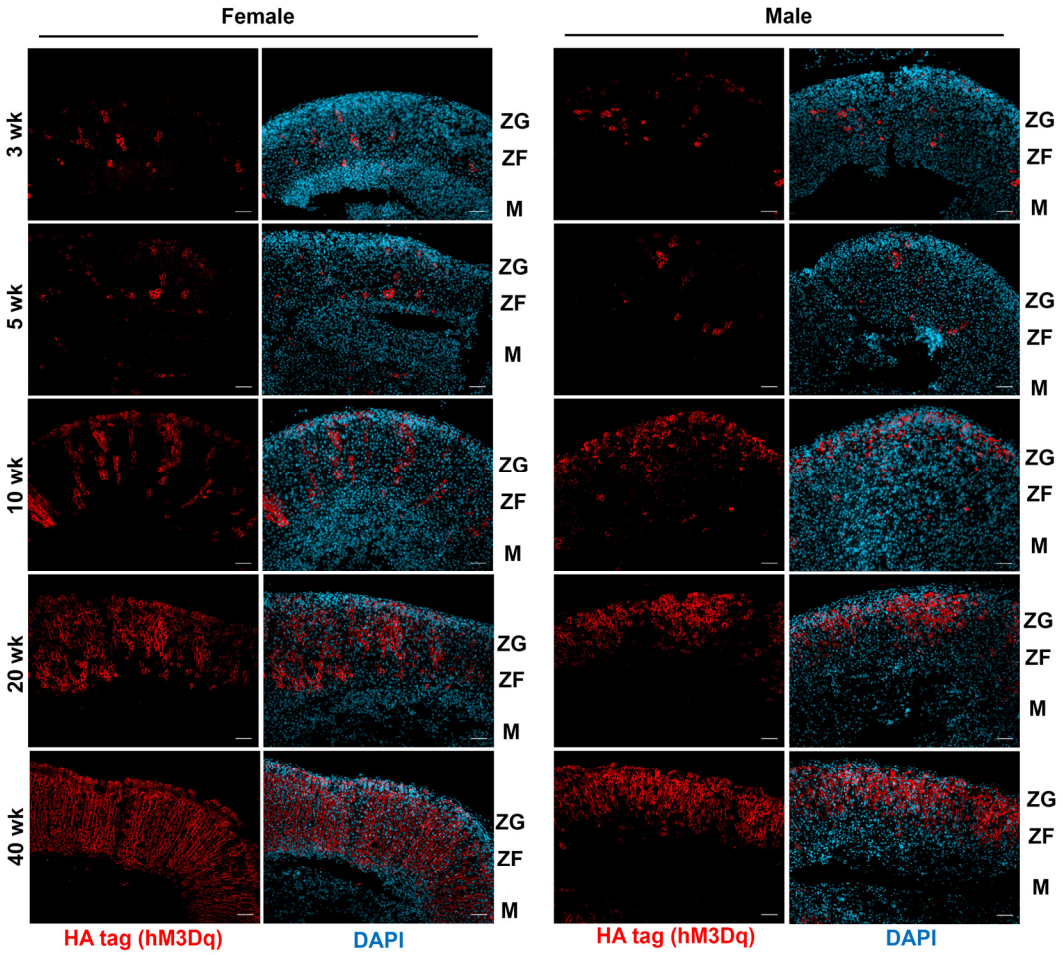
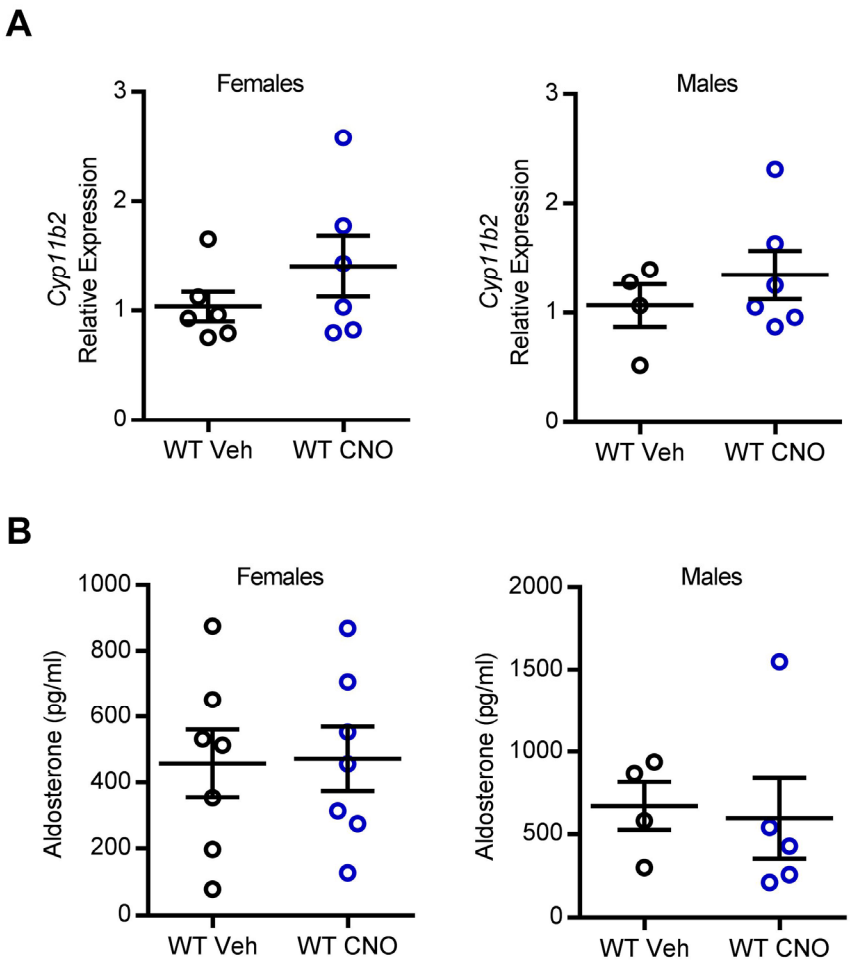


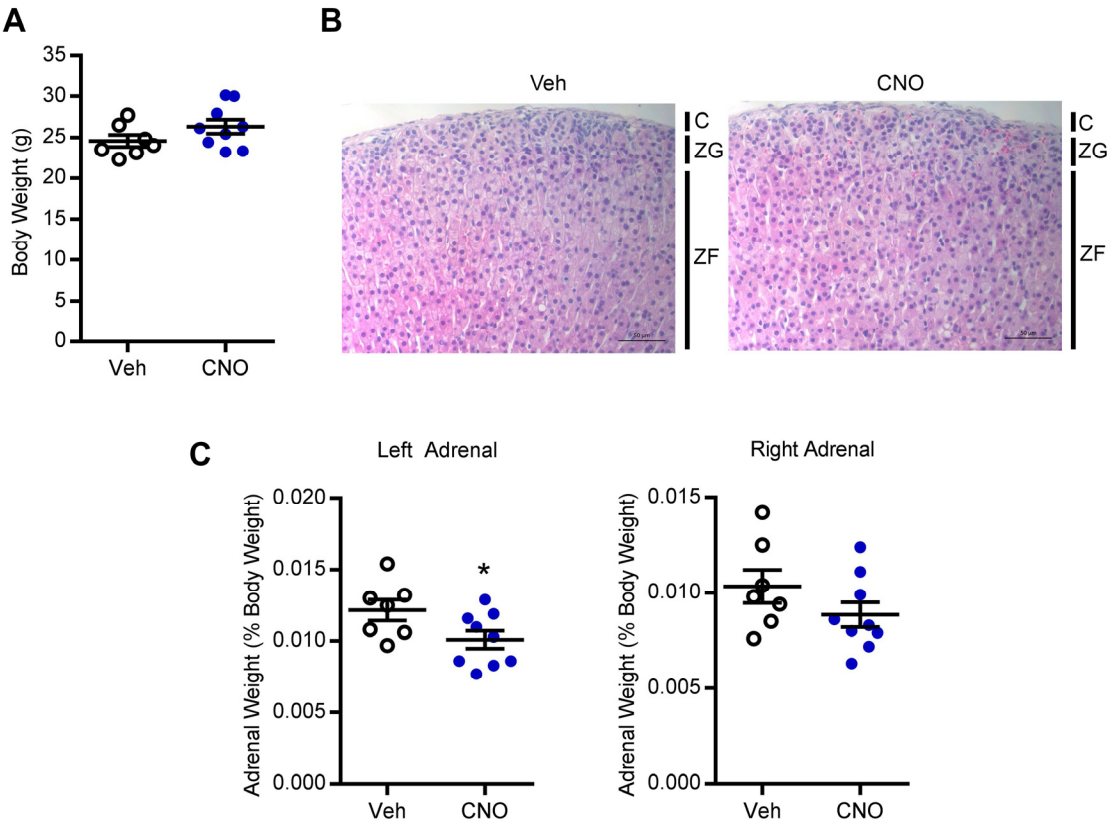
Supplemental Figure 1. Activation of Gq signaling in both the normal and *AS$^{+/Cre}$::hM3Dq* mouse adrenal cells. (A) Angiotensin II (AngII) binding to its receptor (AT_1-R) specifically on the cellular membrane of zona glomerulosa (ZG) cells activates the Gq protein family, leading to *Cyp11b2* transcription and aldosterone production. **(B)** Rationale for the use of DREADD technology. hM3Dq is a modified human muscarinic M3 receptor that binds CNO and not endogenous ligands. This receptor can couple to Gq proteins in the same manner as AT_1-R and activate Gq signaling.



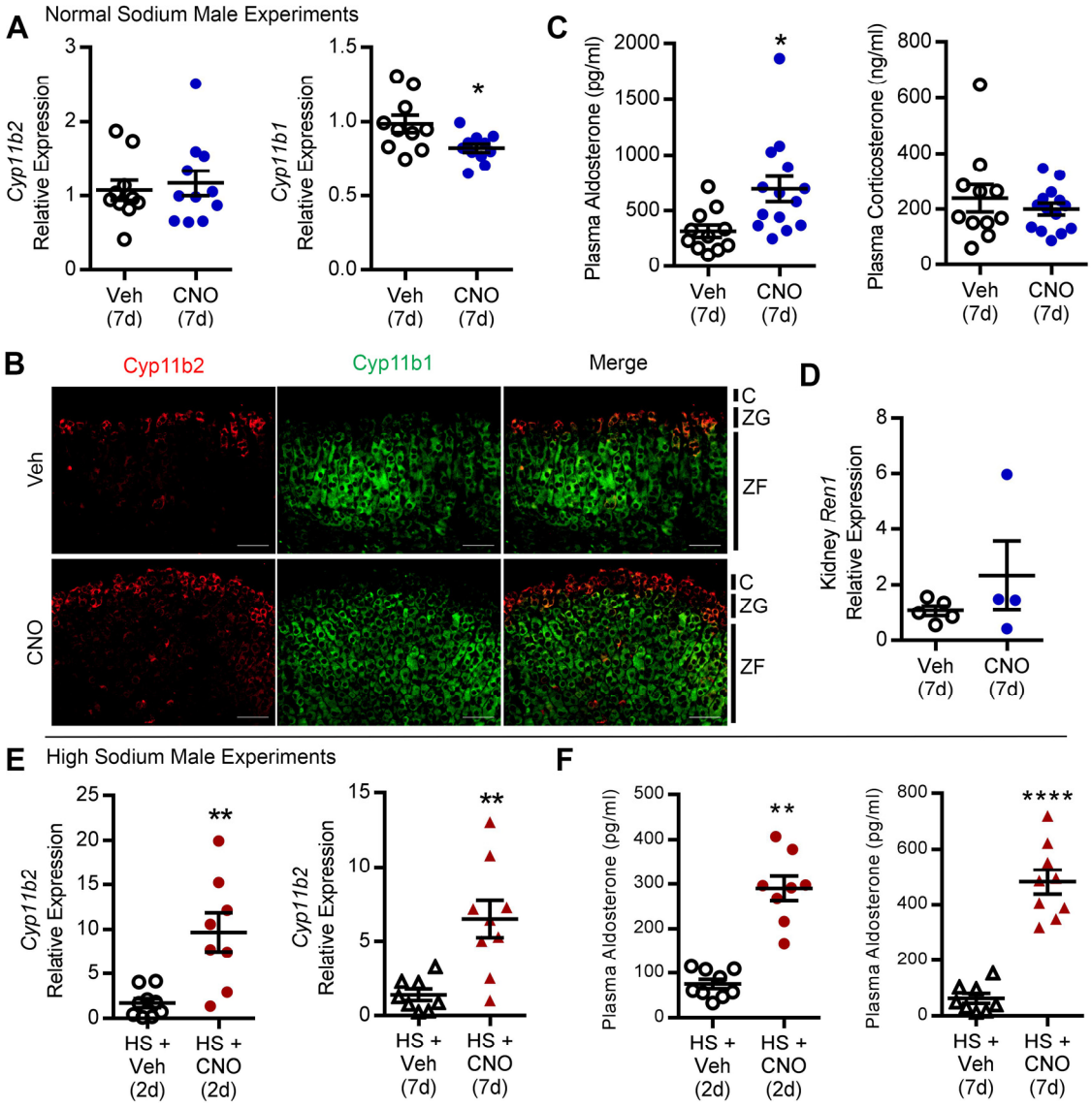
Supplemental Figure 2. Expression of hM3Dq increases with age in the *AS$^{+/Cre}$::hM3Dq* model. Untreated male and female *AS$^{+/Cre}$::hM3Dq* mice were sacrificed at the indicated ages and stained for HA tag (hM3Dq) (red) by immunofluorescence. DAPI (blue) marks the nuclei. ZG, zona glomerulosa; ZF, zona fasciculata; M, medulla. Scale bars = 50 μm.



Supplemental Figure 3. CNO treatment has no significant effect on aldosterone production in wild type mice. Wild type mice (*AS*$^{+/+}$ and either homozygous or heterozygous for *hM3Dq*) were treated with either vehicle or CNO water for 7 days. **(A)** mRNA for whole adrenal *Cyp11b2*. **(B)** LC-MS/MS analysis of plasma aldosterone. For (A), n = 6 for both female groups. In males, n = 4 for Veh and n = 6 for CNO. For (B), in females, n = 7 for both Veh and CNO groups. In males, n = 4 for Veh and n = 5 for CNO. Bars in dot plots represent mean ± SEM. WT, wild type; Veh, vehicle.



Supplemental Figure 4. Additional phenotyping of *AS$^{+/Cre}$::hM3Dq* mice. (A) In 20-21 week old post- treatment females, body weight was measured prior to sacrifice and showed no significant difference between CNO and vehicle. **(B)** The histology of the adrenal glands was examined by H&E staining. **(C)** Adrenal weights were measured and then normalized to each mouse's body weight as a percentage of body weight. For weights, n = 7 for vehicle and n = 9 for CNO. Bars in dot plots represent mean ± SEM. C, capsule; ZG, zona glomerulosa; ZF, zona fasciculata. Scale bars = 50μm. Statistical analysis used for dot plots was unpaired two-tailed Student's *t* test. *$P < 0.05$.



Supplemental Figure 5. CNO treated 20 week old male *AS$^{+/Cre}$::hM3Dq* mice exhibit a moderate response in aldosterone production. (A) Whole adrenal mRNA expression of *Cyp11b2* and *Cyp11b1* in 7 day vehicle (n = 10) or CNO treated (n = 11) males. **(B)** Immunofluorescence of Cyp11b2 (red) and Cyp11b1 (green) in 7 day vehicle or CNO treated males. **(C)** LC-MS/MS steroid measurements in vehicle (n = 11) and CNO treated (n = 14) males **(D)** qPCR analysis of kidney *Ren1* mRNA expression in

vehicle (n = 5) and CNO treated (n = 4) male mice. **(E)** High sodium diet plus CNO *Cyp11b2* expression. 20-22 week old male mice were treated under the same 2 day and 7 day protocols described in Figure 4. **(F)** HS + CNO plasma aldosterone concentration. For 2 and 7 day experiments, n = 9 for both HS + vehicle (2d) and HS + CNO (7d), and n = 8 for both HS + CNO (2d) and HS + vehicle (7d). Bars in dot plots represent mean ± SEM. C, capsule; ZG, zona glomerulosa; ZF, zona fasciculata; Veh, vehicle; 2d, 2 days; 7d, 7 days. Scale bars = 50 μm. Statistical analysis used for dot plots was unpaired two-tailed Student's *t* test. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001.

Sex	Treatment	*n*	11DOC (ng/ml)	Corticosterone (ng/ml)	18OHB (pg/ml)	Aldosterone (pg/ml)
F	Veh (7d)	13	19.3 ± 3.3	342.0 ± 95.1	987.2 ± 145.0	418.8 ± 58.3
	CNO (7d)	17	19.9 ± 4.3	243.4 ± 25.9	2544.5 ± 620.9*	1302.4 ± 265.1**
	HS + Veh (2d)	8	26.4 ± 7.2	288.8 ± 42.8	273.7 ± 54.3	108.8 ± 30.0
	HS + CNO (2d)	8	11.9 ± 4.5	230.3 ± 36.4	488.6 ± 68.5*	285.8 ± 44.9**
	HS + Veh (7d)	8	39.4 ± 9.7	356.4 ± 42.3	110.5 ± 20.4	18.5 ± 5.6
	HS + CNO (7d)	10	20.0 ± 5.0	305.0 ± 28.4	3655.3 ± 1046.0**	1935.2 ± 461.3**
M	Veh (7d)	11	12.4 ± 2.8	239.6 ± 48.5	641.2 ± 128.4	318.2 ± 56.1
	CNO (7d)	14	10.2 ± 2.3	200.5 ± 20.8	1140.5 ± 166.7*	696.1 ± 113.6*
	HS + Veh (2d)	9	23.5 ± 5.2	212.0 ± 27.0	179.5 ± 17.5	75.1 ± 10.2
	HS + CNO (2d)	8	6.0 ± 2.6*	147.4 ± 22.2	466.6 ± 42.7****	289.9 ± 27.6****
	HS + Veh (7d)	8	8.8 ± 2.3	202.5 ± 26.5	170.2 ± 55.0	62.8 ± 17.5
	HS + CNO (7d)	9	6.9 ± 2.4	210.4 ± 30.8	699.7 ± 62.6****	481.9 ± 44.0****

Supplemental Table 1. Concentrations of adrenal steroids in *AS$^{+/Cre}$::hM3Dq* under various treatment protocols.

LC-MS/MS was performed to quantify steroids in mouse plasma from the various treatment protocols. Data for Veh/CNO (7d) corticosterone is presented as dot plots in Figure 4 and Supplemental Figure 4. Data for aldosterone under all conditions is presented as dot plots in Figures 2 and 4, and Supplemental Figure 4. Data is represented as mean ± SEM.

Unpaired two-tailed Student's t-test was performed within each sex/treatment regimen group. *P<0.05, **P<0.01, ****P<0.0001. F, female; M, male; 11DOC, 11-deoxycorticosterone; 18OHB, 18-hydroxycorticosterone; HS, high sodium; Veh, vehicle; 2d, 2 days; 7d, 7 days

Steroid	Steroid Manufacturer	Precursor/ Product Ions (m/z)	RT (min)	LOD (pg/ml)	Internal Standard	Internal Standard Manufacturer
11DOC	Cerilliant	331.2 / 109.0	8.6	11.2	11DOC-d_8	Sigma-Aldrich
B	Cerilliant	347.2 / 329.2	6.8	44.4	B-d_8	C/D/N Isotopes
18OHB	Cerilliant	363.2 / 269.3	4.7	23.0	Cortisol-d_4	Cerilliant
Aldo	Cerilliant	361.2 / 343.1	5.6	3.0	Aldo-d_8	Sigma-Aldrich

Supplemental Table 2. Steroids standards used for LC-MS/MS. m/z, mass to charge ratio; RT, retention time; LOD, limit of detection (calculated by measuring a signal-to-noise ratio of 3). Steroid abbreviations: 11DOC, 11-deoxycorticosterone; B, corticosterone; 18OHB, 18-hydroxycorticosterone; Aldo, aldosterone.

(A) 1260 HPLC Pump - C4 loading column

Mobile Phase A: 0.2 mmol/L Ammonium fluoride (NH_4F)

Mobile Phase B: Methanol + 0.2 mmol/L NH_4F

Time (min)	A (%)	B (%)	Flow (mL/min)	Pressure (bar)
Δ4				
1.00	81.0	19.0	0.500	435
1.20	0.0	100.0	0.500	435
2.50	0.0	100.0	0.500	435
2.51	0.0	100.0	0.000	435
3.90	0.0	100.0	0.000	435
3.91	0.0	100.0	0.100	435
7.20	0.0	100.0	0.100	435
7.40	0.0	100.0	0.100	435
7.41	0.0	100.0	0.500	435
8.99	0.0	100.0	0.500	435
9.00	81.0	19.0	0.500	435
12.00	81.0	19.0	0.500	435
12.10	81.0	19.0	0.500	435

(B) 1290 HPLC Pump - Biphenyl resolution column

Mobile Phase A: 0.2 mmol/L Ammonium fluoride (NH_4F)

Mobile Phase B: Methanol + 0.2 mmol/L NH_4F

Time (min)	A (%)	B (%)	Flow (mL/min)	Pressure (bar)
Δ4				
0.99	81.0	19.0	0.500	480
1.00	55.0	45.0	0.500	480
1.80	55.0	45.0	0.500	480
2.00	40.0	60.0	0.200	480
3.01	40.0	60.0	0.200	480
7.50	23.0	77.0	0.200	460
8.90	23.0	77.0	0.200	460
9.80	10.0	90.0	0.700	600
10.20	0.0	100.0	0.700	600
11.20	0.0	100.0	0.700	600
11.30	81.0	19.0	0.700	600
11.50	81.0	19.0	0.500	600

Supplemental Table 3. Gradient specifications of the Agilent LC system. (A) 1260 HPLC Pump - C4 loading column (B) 1290 HPLC Pump - Biphenyl resolution column.